

## The Activity of Trypsin Inhibitor and its Correlation with the Oil Content in *Glycine*

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### Aktivita trypsin-inhibitoru v semenech některých sort a druhů soje a korelace mezi aktivitou trypsin-inhibitoru a obsahem tuku

1. Byla zjištěna vysoká pozitivní korelace mezi aktivitou trypsin-inhibitoru a obsahem tuku u jednotlivých odrůd a druhů.
2. Korelace mezi aktivitou trypsin-inhibitoru a obsahem tuku uvnitř jednotlivých odrůd nebyla průkazná.
3. Osemení neobsahuje trypsin-inhibitor.
4. Podle aktivity trypsin-inhibitoru a obsahu tuku lze rozdělit sledované formy do 3 skupin, odpovídajících zkoumaným druhům: *Glycine hispida*, *Glycine gracilis* a *Glycine ussuriensis*. Výjimku tvoří odrůda Sioux, která podle sledovaných chemických znaků zapadá do okruhu *G. gracilis*, ačkoliv morfologicky patří mezi *G. hispida*.

### Summary

1. A highly positive correlation has been found to exist between the activity of the trypsin inhibitor and the oil content of some varieties and species of soybean.
2. No correlation could be demonstrated between the activity of the trypsin inhibitor and the oil content within individual varieties.
3. The seed coat contains no trypsin inhibitor.
4. According to the activity of the trypsin inhibitor and the oil content the examined forms can be divided into 3 groups corresponding to the species

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tested: *Glycine hispida*, *Glycine gracilis* and *Glycine ussuriensis*. The variety Sioux forms an exception in conforming to the chemical characteristics of the *Glycine gracilis* group although it belongs to *G. hispida* morphologically.

## Introduction

It is known that the nutritive value of the natural soybean protein is much lower than that of animal protein. The protein efficiency ratio for soybean protein (PER) is 0.5—1.5 (EVERSON and STEENBOCK 1944); the PER value for hen's egg white is approximately 4 (BLOCK and MITCHELL 1946—47). The low PER of soybean protein can be explained in various ways:

- a) by the low content of sulphur-containing amino acids (MITCHELL and BLOCK 1946);
- b) by the presence of toxic soyin (LIENER and PALLASCH 1952);
- c) by the presence of the heat-labile trypsin inhibitor (hereafter designated as TI).

HAM and SANSTEDT (1944) and BOWMAN (1944) independently found a substance in soybeans which inhibits trypsin; KUNITZ (1946, 1947) described its purification and physicochemical characteristics. On the basis of the thermostability of TI (GAITONDE and SOHONIE 1952, FRANĚK 1957) and the increase in the nutritive value of soybeans after heating, it can be deduced that the presence of TI may be one of the most important causes of the low nutritive value of soybean protein.

It was the objective of the present study to find out whether there is any difference in the TI content among the different varieties and species of soybeans and whether there is any correlation between TI and the content of other chemical components of the seeds. An established correlation would facilitate the breeding and selection of varieties with low or high TI contents.

## Material and Methods

For the present work, varieties with large differences in oil and protein content were selected. The majority of the varieties from abroad were not suitable for testing, as the plants had good vegetative growth but did not flower. The Sioux variety, which grows in the most northern parts of the U.S.A. and which is even more matutinal than our varieties, formed an exception. *Glycine ussuriensis* ripened at the same time as our varieties, *Glycine gracilis* ripened very late and had to be transplanted into pots for ripening in a green-house. For a survey of all varieties tested, the time and method of their sowing and the time of flowering and ripening, see GICHNER (1959).

For this experiment the following varieties were used: Kroměřížská žlutá (further Kroměříž), Dieckmanova černá (further D. Č.). Brillmayerova Giesenská (BG2). These varieties were obtained from the Research Institute for Cereal Culture at Kroměříž. The Sioux variety, *Glycine gracilis*, and the wild soybean *Glycine ussuriensis* (uss) were obtained from the Agriculture Research Service, Beltsville, U.S.A. The tested varieties and species were cultivated in the Genetic Garden in Prague in 3 repetitions of 10 test plants. The seeds were ground three times in a spice-mill in order to grind the meal as finely as possible. It was necessary to do this under standard conditions to avoid rather considerable errors in the determination of TI activity.

The soybean meal thus obtained was divided into two parts; one part was used for the determination of TI content, the other for that of oil content.

**Determination of TI content:**

For the determination of TI activity the method of SMETANA, SLAVÍK (1952) in FRANĚK'S (1958) modification was used.

**Preparation of TI extract:**

The soybean meal was extracted in Soxhlet's apparatus with petroleum ether for 4 hours. Amounts of 0.5 g. of the defatted meal were extracted at laboratory temperature for 60 min. in 8 ml. acetate buffer (pH 4). After centrifuging for 5 min., 1 ml. of the extract was made up to 100 ml. with distilled water.

**Preparation of trypsin:**

Fifty mg. crystalline trypsin (Organofarma) was dissolved in 50 ml. 0.01N HCl. One ml. of this solution was diluted to 50 ml. with distilled water before incubation so that 1 ml. contained 20  $\mu$ g. trypsin. This concentration was found to be most suitable under the given incubation conditions.

**Preparation of casein:**

Amounts of 3.7 g. casein (Hammarsten) were dissolved in 95 ml. 0.4N NaOH. Six and 25/100 g. boric acid was added to this solution and the volume made up to 250 ml. with distilled water. The pH of this casein solution was 7.8.

Incubation was carried out in a water-bath at 37° C. Thinwalled test-tubes contained 1 ml. TI solution, 1 ml. trypsin and 1 ml. casein. The incubation period was 30 min. After incubation, the remaining casein was precipitated out of the solution with 3 ml. 5% trichloroacetic acid and after 20 min. the precipitated protein was filtered off. To 3 ml. of the filtrate was added 1 ml. 2N NaOH and 2 ml. Biuret reagent. The colorimetric estimation was carried out in the Lange colorimeter using a VG9 filter. For the blank test 3 ml. distilled water, 1 ml. 2N NaOH and 2 ml. Biuret reagent were used. There was no need to take into account the ballast proteins as the extinction of the blank and of the incubated sample without trypsin (replaced with 1 ml. distilled water) was the same. The amount of uninhibited trypsin was given by the amount of undigested casein as read on the calibration curve. The degree of trypsin inhibition was calculated by subtracting the uninhibited trypsin from 20  $\mu$ g. (the original amount of trypsin). The TI activity was expressed by the degree of trypsin inhibition multiplied by 100 (the extract was diluted 1 : 100). Two tests were made from each plant.

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## Results and Discussion

As it was one of the objectives of the present work to clarify the relation between TI and other chemical characters of soybean, both the TI and the oil content were tested. It was assumed that since TI has the character of a protein it will have a positive correlation with the protein content. On the basis of the negative correlation between the oil content and the protein content of soybean (JOHNSON, ROBINSON and COMSTOCK 1955) a hypothesis was propounded that there will be a negative correlation between the oil content and the TI content,

The TI and the oil content found in different varieties and species are shown percentually in Table 1. The variety BG2 was used as standard  $\bar{x} =$

Table 1. Percentual expression of the activity of the trypsin inhibitor and the oil content of various varieties and species

| Variety, species | Activity of TI | Difference | Oil content | Difference |
|------------------|----------------|------------|-------------|------------|
| D. Č.            | 100.77         | 0.77       | 100.73      | 0.73       |
| BG2              | 100.00         | 1.43       | 100.00      | 15.96*     |
| Kroměříž         | 98.57          | 13.73**    | 84.04       | 8.92*      |
| Sioux            | 84.84          | 0.13       | 75.12       | 0.22       |
| gracilis         | 84.71          | 21.24**    | 74.90       | 40.15**    |
| uss.             | 64.47          |            | 34.75       |            |

The BG2 variety is used as a standard,  $\bar{x} = 1544$  TI activity units,  $\bar{x} = 17.61\%$  oil.

\* Difference significant, for TI values 3.7%, for oil content values 6.3%.

\*\* Difference very significant, for TI values 5.6%, for oil content values 8.8%.

$= 1544$  TI units and  $\bar{x} = 17.61\%$  oil. Analyses of variance showed that the part played by repetitions is insignificant for variability as a whole; the part played by varieties and species, on the contrary, proved to be very significant (see Tables 2 and 3).

The variation coefficient, calculated for the variability within the varieties was higher for TI values (5.99) than for oil values (4.27). This higher variability is caused in great part by methodical errors, as shown by the higher variation coefficient for TI values (0.8) than for oil values (0.38) in analyzing a single sample.

The values found for oil content correspond basically to the data in the literature as far as the relative values are concerned, with the exception of the Kroměříž variety. This variety had an oil content of  $\bar{x} = 14.8\%$ . On analyzing material cultivated at Kroměříž, an oil content of  $\bar{x} = 18.50\%$  was found. Although the analysis was carried out on a mixture of seeds of this variety and although it was not possible to determine the actual variability

Table 2. Analysis of variance (activity of TI)

| Source of variability | N  | $S(x - \bar{x})$ | V       | F       | s      |
|-----------------------|----|------------------|---------|---------|--------|
| Varieties, species    | 3  | 17625140         | 5875046 | 71.857* | 285.93 |
| Repetition            | 2  | 455040           | 227520  | 2.78    |        |
| Error                 | 6  | 490560           | 81760   |         |        |
| Total                 | 11 | 18570740         |         |         |        |

\* very significant

Table 3. Analysis of variance (oil content)

| Source of variability | N  | S(x — $\bar{x}$ ) | V       | F      | s    |
|-----------------------|----|-------------------|---------|--------|------|
| Varieties, species    | 3  | 5938.92           | 1979.64 | 75.18* | 5.13 |
| Repetition            | 2  | 96.14             | 48.07   | 1.82   |      |
| Error                 | 6  | 158.02            | 26.33   |        |      |
| Total                 | 11 | 6193.08           |         |        |      |

\* very significant

lity, the highest values of percentual oil content of the material cultivated in Prague did not reach 18.50% (maximum 16.60%).

On preparing TI from the D. Č. variety it could be seen that a part of the melanin from the seed coat was dissolved in the acetate buffer. It was therefore investigated whether this coloration was not the reason for the apparent rise in TI in the colorimetric estimation. In the control test, beans were used without seed coat and thus prepared, tested for activity. A significant difference was found in the TI activity of soybean meal prepared from beans with seed coats and meal from beans deprived of coats. The extract prepared only out of seed coats did not contain TI. This shows that the dissolved melanin did not affect the values found. The difference in TI content was similarly investigated in soybean meal from *G. ussuriensis* with seed coats and without them (see Table 4). Here it was possible to peel off the seed coat in only 50%, as compared with the variety of D. Č. where the seed coat peeled off completely. Low values of TI activity in the case of *G. ussuriensis* can be explained partly by the fact that the ratio between the seed coats and other parts of the beans was greater than in *G. hispida* and *G. gracilis*.

The correlation between oil content and the TI activity within the BG2 and D.Č. varieties was insignificant:

$$\begin{aligned} \text{variety BG2:} \quad r \pm 3s_r &= 0.0559 \pm 3 \cdot 0.596 \\ t(28) &= 0.09 \quad P > 0.90 \end{aligned}$$

Table 4. The difference between the activity of TI in soybean meal with seed coats and without them

| Variety, species | The difference is very significant in both cases              |                                                            |                       |
|------------------|---------------------------------------------------------------|------------------------------------------------------------|-----------------------|
|                  | Soybean meal without seed coats<br>$\bar{x} \pm 3s_{\bar{x}}$ | Soybean meal with seed coats<br>$\bar{x} \pm 3s_{\bar{x}}$ | Difference            |
| D. Č.            | 1618 $\pm$ 3. 5.82                                            | 1579 $\pm$ 3. 4.48                                         | t(8) = 5.14<br>p 0.01 |
| uss.             | 1036 $\pm$ 3. 11.75                                           | 960 $\pm$ 3. 10.37                                         | t(8) = 4.97<br>p 0.01 |

$$\begin{aligned} \text{variety D. Č.:} \quad r \pm 3s_r &= 0.0246 \pm 3 \cdot 0.586 \\ t(28) &= 0.04 \quad P > 0.90 \end{aligned}$$

The correlation between average values of varieties and species is very significant:

$$\begin{aligned} r \pm 3s_r &= 0.97 \pm 3 \cdot 0.1212 \quad P < 0.01 \\ b_x \pm 3s_{b_x} &= 0.0184 \pm 3 \cdot 0.0029\% \text{ oil/TI unit} \quad P < 0.01 \\ b_y \pm 3s_{b_y} &= 51.71 \pm 3 \cdot 26.47 \text{ TI units/\% oil} \quad P > 0.10 \end{aligned}$$

The difference between the significance in the correlation among varieties and species and the lack of significance within the varieties and species can be explained by the fact that in calculating the correlation between the varieties and species, average values were used which diminished the influence of methodical errors. On calculating the correlation within the varieties the methodical error was possibly so great and the number of analyses so small that a correlation could not be established. The lack of significance in correlation within varieties can also be caused by high variability which masks the existing genotypic correlation. This, however, would not be in accordance with the findings (WEBER and WOORTHY 1952) that in soybeans the genotypic correlation is higher than the phenotypic one in the majority of characters. Authentic conclusions could only be reached if a genetic analysis were carried out and values determined for a genotypic and a phenotypic correlation.

In generalizing the results we must, in view of the low number of tested varieties and species, take the strikingly high value of the correlation coefficient with certain reserve. If correlation had been calculated only within the varieties of *G. hispida*, it would probably not have been so high, nevertheless, such a high correlation is rather surprising.

According to its oil content and TI activity the material tested can be divided into three groups:

- a) with high oil content and TI activity — BG2, D. Č, Kroměříž;
- b) with average TI activity and low oil content — Sioux, *G. gracilis*;
- c) with low TI activity and minute oil content — *G. ussuriensis*.

These groups correspond to the 3 examined species: *G. hispida*, *G. gracilis*, *G. ussuriensis*, only the Sioux variety belongs to the *G. gracilis* group as far as its chemical characters are concerned but it belongs to *G. hispida* morphologically.

The original hypothesis that there is a positive correlation between the oil content and the TI activity was proved to be false.

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### Активность трипсин-ингибитора в семенах некоторых сорт и видов сои и корреляция между активностью трипсин-ингибитора и содержанием жира.

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1. Установлена высокая положительная корреляция между активностью трипсин-ингибитора и содержанием жира у отдельных сортов и видов.
2. Корреляция между активностью трипсин-ингибитора и содержанием жира в пределах отдельных сортов не является достоверной.
3. Оболочка семени не содержит трипсин-ингибитор.
4. По активности трипсин-ингибитора и содержанию жиров возможно изученные формы разделить в три группы, отвечающие изученным видам: *Glycine hispida*, *Glycine gracilis* и *Glycine ussuriensis*. Исключение составляет сорт Sioux, который по изученным химическим свойствам принадлежит в диапазон *G. gracilis*, хотя морфологически принадлежит к *G. hispida*.