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Features of Determining Trypsin Inhibitors' Activity in Soybeans by Using the Casein Method

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Abstract

The conducted studies have identified some of the features of the "casein method" that determines the activity of trypsin inhibitors in soybeans. Trypsin inhibitors are anti-nutritional substances that limit the use of soybean for food and feed purposes, so the constant control over their content is required. As a result of research, it was found that the most effective wavelength for spectrophotometric determination of casein decay products, the number of which characterizes the activity of inhibitors, is 276 nm, and the optimal extract amount of the protein extraction required for analysis should be within 0,35 – 0,40 ml.

Keywords: Soy seeds, trypsin, inhibitory, activity, casein method, spectrophotometer, decay product.

INTRODUCTION

The disadvantage of soybean as a food and feed crop is the availability of protein nature substances in its seeds that inhibit the activity of digestive enzymes, in particular trypsin. *Trypsin is an enzyme of hydrolases class that breaks up peptides and proteins in the gastrointestinal tract of humans and animals.* When using raw soybean, the digestive system of humans and live-stock animals is inhibited. In this regard, prior to use, soybeans and products of its processing are subjected to various treatments in order to reduce this negative effect with constant control over the content of inhibitors.

Proteolytic enzymes inhibitors are substances of protein origin and represented by lectins, urease, lipoxygenase and some other compounds [6]. In soybean grain, trypsin inhibitors are accounted for 5-10% of the total protein content. They accumulate mainly in cotyledons and they are practically absent in the leaves, stalks, roots and shell of the beans.

Soybean protease inhibitors are divided into two categories: with a molecular weight of 20000 to 25000 atomic mass units (a.m.u) and with a small number of disulfide bridges (Kunitz inhibitor), and with a molecular mass of 6000-10000 a.m.u. and with a large number of disulfide bridges (Bauman-Birk inhibitor). Just exactly the disulfide bridges are able to inhibit trypsin. The result of the joint effect of Kunitz and Bauman-Birk inhibitors is a decrease in protein assimilability by 4–5 times [3].

A very low correlative relationship between protein content and the value of trypsin inhibitory activity (TIA) (r = 0, 22) is noted in the literature [2,4]. The activity of protease inhibitors varies greatly in soybean seeds and depends on growing conditions and genotype. The dependence of TIA on the size of the seeds is marked: the greater the mass of 1000 seeds, the less TIA [6].

Among other legumes, soybean has the highest trypsin inhibitory activity [7]. Despite the fact, that the development of methods for determining trypsin inhibitors has been going on for many years [8,9], it is important to improve these technologies, especially for soybean. Currently, the determination of trypsin inhibitors activity in legumes seeds is quite often carried out by the “casein method”, which was developed by Kakada and later modified by I.I. Benken [1,8]. One of the conditions responsible for the accuracy and the results reproducibility of the "casein method" is the determination of TIA at this degree of inhibition of trypsin in the incubation mixture, when its activity decreases in proportion to the amount of added extract. It is known that different types of legumes do not always maintain a linear dependence between these values [1]. This dependence is not established for soybean. The goal of research is to study the features of the casein method, in relation to soybean.

MATERIALS AND METHODS

The objects of research were dry soybean seeds of new and promising soybean varieties selected by the FSBSI ARSRI of Soybean (Amur region, Russia). The seeds were grinded up and the flour was defatted by petroleum ether. Extraction of inhibitors was carried out with borate buffer (pH = 7,6). Portion of the extract was added to a mixture of casein and trypsin and incubated at 37°C. After incubation, the amount of casein decay products was determined spectrophotometrically. The inhibitor activity was expressed in the amount of trypsin, spent on the formation of casein decay products (mg/g).

The “casein method” is based on a photometric determination of the number of inhibited trypsin units - casein decay products. At the same time, one unit of trypsin is arbitrarily defined as an increase in extinction (light absorption) by 0,01 at 260 – 280 nm. Since the determination is relatively “arbitrary”, so each trypsin batch requires a

correction of the wavelength used in spectrophotometric measurements. To establish the optimal parameters, an absorption spectrum of trypsin solution was taken on a Cary 50 Scan spectrophotometer (Varian Company) in the ultraviolet range.

RESULTS AND DISCUSSIONS

Figure 1 shows the absorption spectrum of trypsin (solution of dry trypsin (12, 5 mg /100 ml) from the pancreas of a pig, for cell culture, by BioloT Company, Russia).

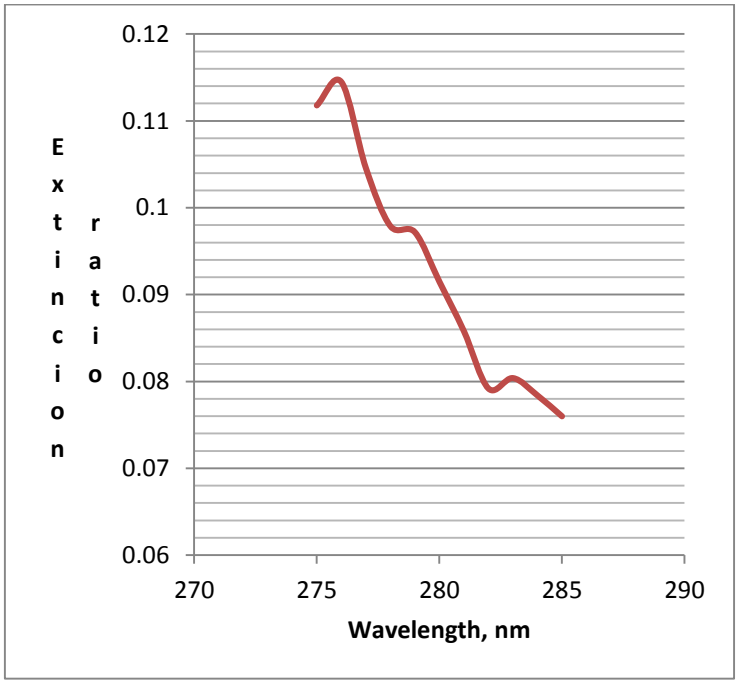

Figure 1. Absorption spectrum of trypsin.

As can be seen from the spectrum shown in Figure 1, the optimum wavelength for trypsin of the given batch is 276 nm, which was also used in further measurements, despite the fact that the methodological guidelines usually recommend to carry out measurements at 280 nm [1].

Figure 2 presents the dependence of the extinction ratio of the trypsin inhibition reaction on the volume of extract used for incubation. In accordance with the obtained results, when studying the activity of Kunitz inhibitor in the soybean seeds, the volume of extraction, required for the carrying out reaction, should be in the range of 0,35-0,4 ml.

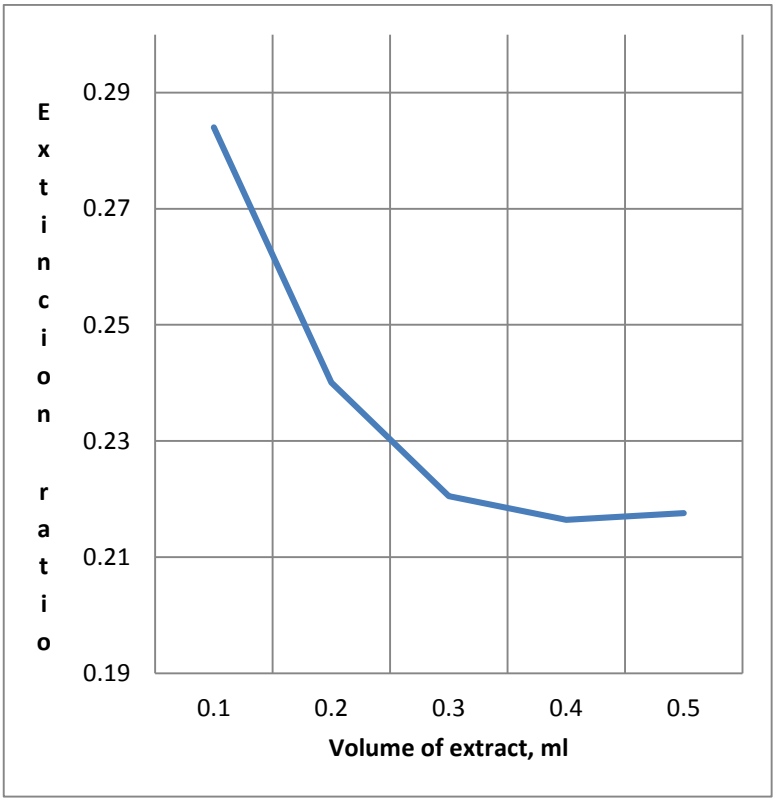

Figure 2. Inhibition of trypsin activity.

If the above parameters are observed (the wavelength is 276 nm, the volume of extraction is 0, 35 ml), the trypsin inhibitory activity of some soybean varieties is established (Table 1). The activity of trypsin inhibitors in soybean seeds varies widely that confirms the need for careful control of soy products for food and feed purposes.

Table 1. Trypsin inhibitory activity of some soybean varieties.

Soybean variety	TIA, mg/100 g of flour
Kitrosa	13,64±0,75
Alena	19,94±0,85
Ariika	31,95±0,86

As a result of the conducted research it has been found that while using the "casein method" in determining the activity of trypsin inhibitors, the most effective wavelength at the spectrophotometric determination of casein decay products is 276 nm, the volume of extraction of the protein extract from soybean seeds should be within 0,35 – 0,4 ml.

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