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THERMAL INACTIVATION OF THE HEMAGGLUTINATING ACTIVITY IN EXTRACTS OF DIFFERENT BEAN CULTIVARS (*Phaseolus vulgaris*)

TOPLINSKO ONESPOSOBLJAVANJE AKTIVNOSTI AGLUTINACIJE U EKSTRAKTU RAZNIH KULTIVARA GRAHA (*Phaseolus vulgaris*)

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SUMMARY

Agglutination activity and partially heat stability of different cultivars of kidney beans (*Phaseolus vulgaris*) can be determined by using the hemagglutination test. Rabbit and human red blood cells and BHK cells are the most sensitive for detection of kidney bean lectin. The highest agglutination activity has been detected in red kidney cultivar (45,5 HE mg^{-1}) and in the cultivars of processor cipro and jeruzalemčan (17,3 HE mg^{-1}). Heating presoaked beans at 100°, 85° or 70°C for 15, 30 or 60 min. decreased the agglutinating activity. PHA stayed active even after being cooked for 60 minutes at 70°C. Almost the whole amount of lectines was inactivated after being cooked for 10 minutes at 100°C.

INTRODUCTION

Among the many antinutrients present in plant food there is a group of partially heat stable glicoproteins or lectins. These are carbohydrate-binding proteins of non-immune origin capable of specific recognition of, and reversible binding to, carbohydrates without altering their covalent structure (Kocourek and Horejsi, 1983). Nachbar and Oppenheim (1980) found evidence of the presence of lectins in 53 edible plants. These antinutritive glycoproteins are usually found in high but variable concentrations in legume seeds and they also occur in bark, roots, fruit or leaves of different plant species. One of its richest sources are kidney beans (*Phaseolus vulgaris*) which represent an important component in animal and human diets. Furthermore, different cultivars of the same species, e.g. cultivars of *Phaseolus vulgaris*, can differ markedly in their lectin content and toxicity. For example, in kidney beans, lectins (phytohemagglutinins) represent about 15-20% and in soybeans, about 1% of the total amount of proteins (Baintner, 1993). Foods which contain low levels of lectins, however, are not toxic when eaten. Beans in their wet immature state, such as green bean in their pods, contain small amounts of lectins. In addition lentils, garden peas, and split peas also contain a relatively small amount of lectins (McPherson, 1989).

Lectins can have dramatic effects on the entire digestive tract and its bacterial population and on metabolism. Their extraordinary effectiveness stems from their resistance to proteolysis, coupled with high and specific chemical reactivation with endogenous surface receptors of the epithelial cells

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of the gut (Pusztai, 1993): Lectins are powerful oral and parenteral immunogens and some of their physiological effects are intricately linked to their interference with immune function. However, the primary effects and the potency of lectins as biological signals are a direct reflection of their specific chemical reactivity with saccharides (Pusztai, 1991).

The way to detect the presence of lectins in biological material is to prepare a water extract from this material and to examine its ability to agglutinate different blood cells and cell lines. Hemagglutination is usually determined by the serial dilution technique. Hemagglutination titres are the best semi-quantitative and subject to critical evaluation (Bonorden and Swanson, 1992).

Few studies are available on the thermal inactivation of hemagglutinating activity (HA) of kidney bean lectin. PHA is partially stable in respect to thermal treatment (Paredez-Lopez, et all, 1989). Therefore, there is a concern if the beans are not sufficiently cooked, as it sometimes is the case when prepared at high elevations or in low temperature processes (Noah, 1980). *In vitro* hemagglutinating assay indicated that the toxin in red kidney beans was completely destroyed by about 10 min. boiling (Thompson, 1983). However, when the same samples were heated below 100°C the activity was slowly reduced, especially within the first 15 min., and it was not completely eliminated (Paredez-Lopez et al., 1989). Heating at 80°C increased the hemagglutinating activity about five fold, implying that incompletely cooked red kidney beans may be more toxic than the raw ones (Coffey et all, 1992). Raw, or poorly cooked kidney beans caused diarrhoea, depression of growth, weight loss and death in experimental animals (Coffey et all, 1985).

This study was undertaken to determine the conditions of time and temperature required to destroy the hemagglutinating activity in different cultivars of kidney beans.

MATERIAL AND METHODS

Bean samples

Samples of different kidney bean (*Phaseolus vulgaris*) cultivars were used. First. Slovenian cultivars cipro, jeruzalemčan and lusia. Second, red kidney, pinto and mungo beans provided by the Kmetijski Institut (Ljubljana, Slovenia). Third, processor sample provided by the Rowett Research Institute (Aberdeen, Scotland).

Preparation of bean extracts for hemagglutinating activity determinations

The extracts were prepared from seeds ground in a mill (Gorenje, Slovenia). The ground material was mixed with ten times the amount of 0.9% physiological saline (beans: saline, 1:10, w/v) and soaked overnight at 4°C. After soaking, the ground beans were subjected to various heat treatments (100°, 85° or 75°C) and times (15, 30 or 60 minutes), and clarified by centrifugatin (10,000 x g, 30 min.). The saline supernatant was decanted and assayed for hemagglutinating activity either on the same day or frozen and assayed on the following day.

Preparation of the red blood and culture cells

Rabbit and human blood (group 0) was collected into preheparinized tubes, and after collection washed three times with 0.9% physiological saline and diluted twenty fold with saline (Jaffe et al., 1972). The sensitivity of the red blood cells to agglutination was increased by treatment with 0.25% trypsine from porcine pancreas, type IX (Sigma). Following incubation at 37°C for 30 min., the treated cells were washed three times with saline and either prepared 1% suspension of red blood cells in saline for the hemagglutination assay or packed by centrifugation (400 x g, 10 min.) and stored at 4°C for later use. Treated and packed cells were stable at 4°C for up to 5 days.

Transform baby hamster kidney fibroblast cell line (BHK) was used in our studies. Those cells were routinely grown in Dulbeco's modified enriched medium (DMEM) (Sigma) supplemented with 10% foetal calf serum, antibiotics and L-glutamine in humidified atmosphere of 95% air and of 5% CO_2 at 37°C in Falcon tissue culture flasks. The cells were removed from their grown vessels using 0.25% trypsine in Hanks buffer (Ca^{2+} and

Mg^{2+} free) and incubated for 10 min. at 37°C. The flask was rinsed twice with DMEM medium and cells resuspended in the 0.9% physiological saline at concentration of $9.92x10^5$ cells ml^{-1}.

Hemagglutinating assay

Hemagglutinating assay was performed in v-bottom microtiter plates for red blood cells and in u-bottom microtiter plates (Falcon Primaria, New Yersey, USA) for cell line. Starting with 50 µl of the extract, serial twofold dilutions were made with 0.9% physiological saline (Felsted et al., 1975). Trypsinized rabbit or human red blood cell or cell culture suspensin (50 µl) was then added. The plates were covered to prevent moisture evaporation and shaken on platform shaker for 10 min. After 1 hr incubation at 37°C, agglutination patterns were observed with the aid of microscope. A concentration of 10 mg ml^{-1} of purified lyophilised PHA (Sigma) in saline was made daily as a standard. One unit of hemagglutination activity was defined as the amount of material per ml in the last dilution giving 50% hemagglutination. Results were represented in hemagglutinating units (HU) per mg of dry weight (DW) (Fish and Thompson, 1991).

RESULTS AND DISCUSSION

The highest sensitivity for detection of lectins in kidney bean extracts was achieved with trypsinized rabbit red blood cells and the BHK cell line (Table 1). The highest hemagglutinating activity was detected in the red kidney cultivar, i.e. 45.52 HE mg^{-1} of dry weight of bean material. Lower activity was detected in the cultivars of processor, cipro and jeruzalemčan, 17,3 HE mg^{-1}, and lusia, 11,4 HE mg^{-1} of dry weight (Table 1). It can be concluded that Slovenian bean cultivars, i.e. cipro, jeru-zalemčan and lusia contain high amounts of PHA. As expected, the lowest activity was detected in the cultivars of pinto and mung with 1.42 and 0.36 HE mg^{-1} of dry weight, respectively.

Changes in HA of the extracted PHA heated at various temperatures and times are shown in Table 1. In a simple thermal process, such as cooking, their activity decreases with increased temperature and heating time. PHA becomes sensitive to heat at temperatures above 70°C. However, temperatures lower than 100°C do not completely inactivate its activity (Fig. 2. and 3).

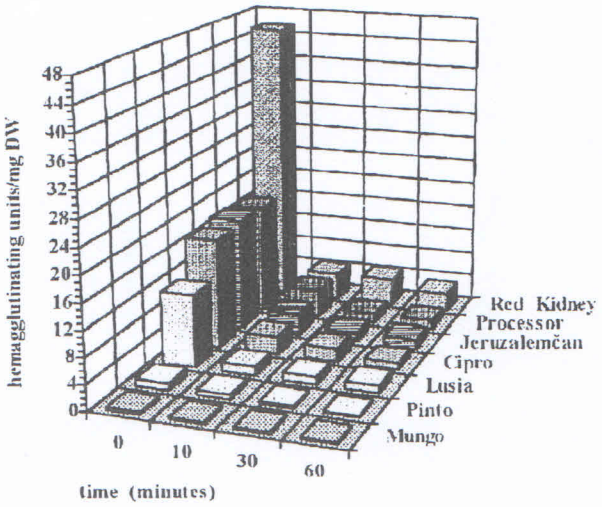

Figure 1: Thermal inactivation of hemagglutinating activity of PHA extracted from different kidney bean cultivars (cooked at 70°C for 10, 30 or 60 min., respectively) determined with the cell line

Slika 1: Toplinsko onesposobljavanje hemaglutinativne aktivnosti PHA ekstrahiranog iz raznih kultivara običnog graha (kuhanog na 70°C kroz 10, 30 ili 60 min.) određenog staničnom linijom

In our experiments, we have established that hemagglutination activity in all samples, especially in those with a high amount of PHA, was significantly reduced in first 10 minutes of thermal treatment. PHA in kidney bean extracts that were treated at 70°C was still active after 60 min. cooking (Table 1). After cooking at 70°C, the red kidney bean cultivar extracts contained 6% of active lectin amount in raw beans, the cultivars of processor, cipro, jeruzalemčan and lusia contained 8-9%, pinto 26%, and mungo 61%, respectively. The presence of detectable levels of HA following long holding times at low cooking temperatures indicated that the possibility existed for the chronic consumption of low levels of PHA in the diets of consuming populations.

Table 1: Hemagglutinating activity of extracts of different kidney bean cultivars processed at different temperatures and times according to various red blood cells and the BHK cell line

Tablica 1: Hemaglutinativna aktivnost ekstrakata raznih kultivara običnog graha obrađenog na raznim temperaturama i trajanja prema raznim crvenim krvnim zrncima i staničnim linijama BHK.

Cooking temperature Temperatura kuhanja		70°C			85°C			100°C		
time (minutes) vrijeme	0	10	30	60	10	30	60	10	30	60
Red kidney										
eri-h	45.52	4.27	3.56	2.13	0.89	0.35	0.13	0.18	0.03	0.02
eri-r	45.52	5.69	5.69	4.27	2.84	0.53	0.27	0.18	0.11	0.02
BHK	45.52	4.27	4.27	2.84	1.07	0.20	0.11	0.07	0.07	0.02
Procesor										
eri-h	11.46	4.27	1.07	1.07	0.13	0.10	0	0.03	0.01	0
eri-r	17.31	2.85	1.42	1.42	0.17	0.18	0.02	0.04	0.02	0.01
BHK	17.31	4.25	1.42	1.42	0.09	0.07	0.02	0.03	0.01	0.01
Jeruzalemčan										
eri-h	11.81	2.20	0.92	1.65	0.27	0.18	0.10	0.18	0	0.01
eri-r	11.81	2.20	1.47	1.47	0.37	0.27	0.14	0.37	0.04	0.02
BHK	17.82	3.30	1.47	1.47	0.18	0.14	0.18	0.14	0.02	0.01
Cipro										
eri-h	11.46	1.42	1.07	0.71	0.41	0.11	0.13	0.18	0.04	0.02
eri-r	14.24	2.85	1.42	1.07	0.36	0.11	0.13	0.36	0.04	0.09
BHK	17.30	2.85	2.14	1.07	0.44	0.09	0.09	0.10	0.03	0.02
Lusia										
eri-h	11.43	0.71	1.59	0.79	0.35	0.18	0.13	0.09	0.04	0.02
eri-r	11.43	1.42	1.59	2.13	0.35	0.18	0.18	0.09	0.04	0.02
BHK	11.43	1.42	1.07	1.06	0.44	0.39	0.20	0.05	0.04	0.01
Pinto										
eri-h	1.06	0.53	0.53	0.71	0.09	0.04	0.03	0.04	0.02	0.01
eri-r	1.42	0.71	0.71	1.06	0.11	0.04	0.04	0.04	0.01	0.01
BHK	1.06	0.71	0.27	0.35	0.11	0.05	0.02	0.03	0.03	0
Mungo bean										
eri-h	0.36	0.36	0.22	0.13	0.18	0.13	0.09	0.09	0	0
eri-r	0.36	0.27	0.27	0.18	0.27	0.13	0.09	0.13	0.02	0.01
BHK	0.36	0.27	0.27	0.22	0.13	0.19	0.10	0.11	0.04	0.03

eri-h=human red blood cells - crvena krvna zrnca čovjeka, eri-r=rabbit red blood cells - crvena krvna zrnca kunića, BHK=baby hamster kidney fibroblast cells - stanice fibroblasta bubrega mladih zamoraca

The values are the means of three determinations on each cultivar.

Vrijednosti su prosjek od tri pretrage svakog kultivara.

HA is expressed as hemagglutinating unit mg^{-1} or dry weight bean material.

HA je prikazan kao hemaglutinska jedinica mg^{-1} ili suha težina graha.

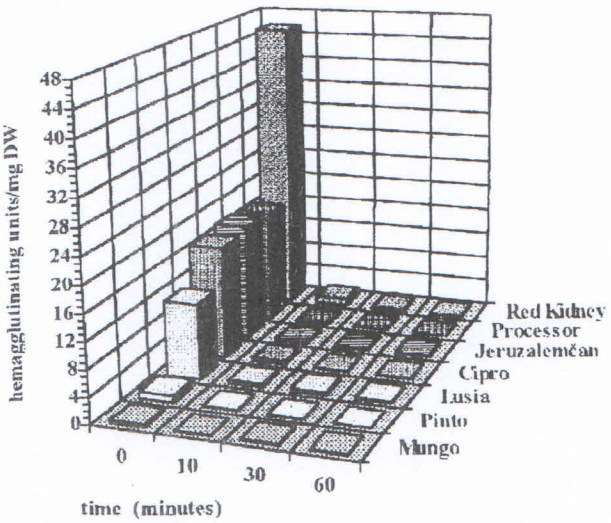

Figure 2: Thermal inactivation of hemagglutinating activity of PHA extracted from different kidney bean cultivars (cooked at 85°C for 10, 30 or 60 min., respectively) determined with the cell line

Slika 2: Toplinsko onesposobljavanje hemaglutinativne aktivnosti PHA ekstrahiranog iz raznih kultivara običnog graha (kuhanog na 85°C kroz 10, 30 ili 60 min.) određenog staničnom linijom

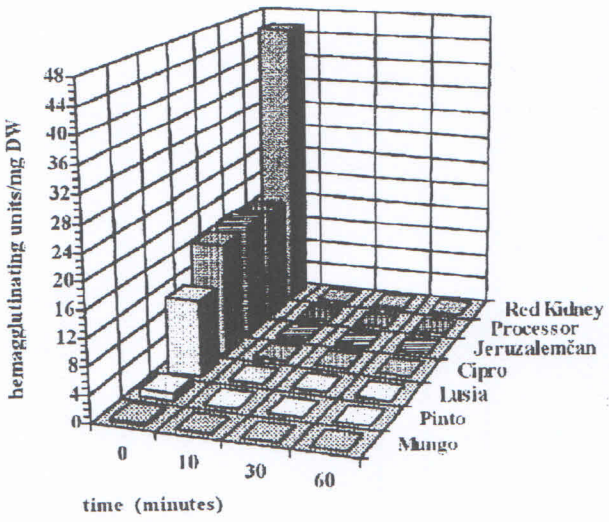

Figure 3: Thermal inactivation of hemagglutinating activity of PHA extracted from different kidney bean cultivars (cooked at 100°C for 10, 30 or 60 min., respectively) determined with the cell line

Slika 3: Toplinsko onesposobljavanje hemaglutinativne aktivnosti PHA ekstrahiranog iz raznih kultivara običnog graha(kuhanog na 100°C kroz 10, 30 ili 60 min.) određenog staničnom linijom

Cooking samples at 100°C successfully reduced the amount of toxic PHA after 10 minutes (Fig. 1). Having been cooked for 60 minutes at 100°C, in all but one samples only 0.01-0.03 HE mg^{-1} of dry weight of PHA was detected (Fig. 1). The only exception was pinto cultivar where the activity of PHA was completely inactivated (Table 1).

CONCLUSIONS

Hemagglutinating activity of PHA is a function of lectin concentrations and varies among different cultivars. It can be concluded that Slovenian bean cultivars, i.e. cipro, jeruzalemčan and lusia, contain high amounts of PHA. The majority of PHA activity is eliminated from presoaked beans by heating at 100°C for 10 min. However, hemagglutinating activity was detected in cultivars exposed to low temperatures (70° and 85°C) for 60 min. These data do not indicate the nutritional quality of beans cooked at low temperatures but may have important nutritional implications. Further research is needed to evaluate any nutritional or physiological impairment resulting from chronic consumption of bean diets containing low levels of active PHA.

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SAŽETAK

Aktivnost aglutinacije te djelomično toplinska stabilnost raznih kultivara graha (Phaseolus vulgaris) mogu se odrediti primjenom testa hemaglutinacije. Crvena krvna zrnca kunića i ljudi te BHK stanice najosjetljivije su na otkrivanje lektina graha. Najjače djelovanje aglutinacije otkriveno je u kultivaru crvenog graha (45,5 HE mg^{-1}) te u kultivarima processora, cipro i jeruzalemčane (17,3 HE mg^{-1}). Grijanje je prethodno namočenog graha na 100°, 85° ili 70°C kroz 15, 30 ili 60 min. smanjilo djelovanje aglutinacije. PHA ostaje aktivnom čak i nakon kuhanja od 60 minuta na 70°C. Gotovo su sve količine lektina postale nedjelotvornima nakon kuhanja od 10 minuta na 100°C.