

## THE INVESTIGATION OF COAGULATION ACTIVITY OF NATURAL COAGULANTS EXTRACTED FROM DIFFERENT STRAINS OF COMMON BEAN

*Marina B. Šćiban, Mirjana A. Vasić, Jelena M. Prodanović, Mirjana G. Antov and Mile T. Klačnja*

*Coagulation and flocculation by adding chemicals are the methods that are usually used for removal of water turbidity. This study is concerned with the coagulation activity of extracts of various strains of bean. The aim was to ascertain if bean varieties influence coagulation activity. Active components were extracted from 1 g of ground sample with 100 ml distilled water. Contents of dry matter and nitrogen were specified in the solid samples, and the content of soluble nitrogen was determined in the extracts. These data were used to calculate the efficiency of extraction of nitrogen-containing compounds. The coagulation activity was assessed by jar test using synthetic turbid water, of the initial pH 9 and turbidity 35 NTU. The jar test was carried out by adding different amounts of extracts to model water, and stirring the content. After sedimentation for 1 h, residual turbidity was determined by turbidimeter and coagulation activity was calculated. The increment of organic matter concentration after the coagulation was also determined. These experiments confirmed that extracts of all investigated strains of bean could be used successfully as natural coagulants.*

KEYWORDS: Water clarification, natural coagulants, various strains of bean

### INTRODUCTION

Natural water is usually turbid to some extent. Coagulation and flocculation are commonly used methods for water turbidity removal, and are usually conducted by adding chemicals such as salts of aluminium and iron and polyelectrolytes. The first investigations about harmful influence of these chemicals on human health were published in the 60's of the 20th century. Those and later publications showed that the residues of aluminium salts in the water can cause Alzheimer's disease (1, 2, 3). Also, there are studies that indicate that some of synthetic organic polymers, such as acrylamide, have strong neurotoxic and carcinogenic effects (4).

---

Dr. Marina B. Šćiban, Assist. Prof., Jelena M. Prodanović, M.Sc., Res. Assist., Dr. Mirjana G. Antov, Assist. Prof., Dr. Mile T. Klačnja, Prof., University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Republic of Serbia, Dr. Mirjana A. Vasić, Sen. Res. Assoc., Inst. of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

In view of the above problems, intensive investigations of natural coagulants have been conducted in the last years in order to replace chemical coagulants in water treatment. It is believed that natural coagulants, that can originate from plants, animals or microorganisms, are not harmful, and besides, the resulting biodegradable sludge can be disposed in the nature without any adverse influence.

The idea of water clarification by natural coagulants is many centuries old. There are written documents from India in which the seed of *Strychnos potatorum* tree was mentioned as water clarifier (5). In the XVI and XVII century, militaries of Peru used roasted and ground corn beans (*Zea mays*) for this purpose. Recently, the most investigated plant is *Moringa oleifera*, whose ground seeds are used for water clarification by women in rural areas of Sudan. Results of these investigations confirmed that *Moringa oleifera* seed extract is very efficient for water clarification (6, 7).

Considering the fact that *M. oleifera* is a plant that originates from tropical areas, we wanted to investigate the possibility of preparing natural coagulants from sources that are cheap and easily available in this region. Our previous investigations confirmed the fact that extracts of various strains of Leguminose could be used as natural coagulants (8). The aim of this study was to obtain natural coagulants from different strains of bean, in order to compare their coagulation activity.

## EXPERIMENTAL

**Model water.** The coagulation activity was assessed by jar test using synthetic turbid water. As first, kaolin was ground in a ceramic mortar and sieved through the sieve with pore size of 0.4 mm. Smaller fraction was then taken to prepare a 10 g/l suspension in tap water. The suspension was stirred for 60 minutes on a magnetic stirrer, and left for 24 hours in order to achieve complete hydration of kaolin. Model water was prepared just before performing the coagulation test, by adding this 1% kaolin suspension to tap water in an amount of 5 ml/l to obtain the water with initial turbidity of 35 NTU (nephelometric turbidity units).

**Coagulants.** Natural coagulants were extracted from four strains of bean, cultivated in the Institute of Field and Vegetable Crops, Novi Sad:

- Sample 1 – Levač bean
- Sample 2 – Sremac bean
- Sample 3 – Zlatko bean
- Sample 4 – Balkan bean.

Natural coagulants were obtained in the following way: seeds were ground and sieved through the sieve with pore size of 0.4 mm. An amount of a 10 g/l of the smaller fraction was suspended in distilled water. This suspension was stirred 10 minutes on a magnetic stirrer in order to extract active coagulants. After that, the suspension was filtered through filter paper Macherey-Nagel MN 651/120. Obtained filtrates, called crude extracts, were stored in a refrigerator at 5°C.

Contents of dry matter and nitrogen were specified in the solid samples, and the content of soluble nitrogen was determined in the extracts. These data were used to calculate the efficiency of extraction of a nitrogen-containing compounds.

**Analytical methods.** Dry matter was determined by standard method at 105°C (9). Content of nitrogen in ground samples and crude extracts was determined by the Kjeldahl method (9). The pH was measured using a pH meter. Water turbidity was measured by nephelometric method on a turbidimeter (10) and expressed in NTU. Content of organic matter before and after coagulation was determined as permanganate demand (10).

**Coagulation test.** The coagulation activity was assessed by jar test using synthetic turbid water, with the kaolin concentration of 50 mg/l and turbidity 35 NTU. The pH of the model water was adjusted to pH 9 by adding 1 mol/l NaOH just before performing coagulation test. The jar test was carried out by adding different amounts of extracts to 300 ml of model water. Fast stirring at 200 rpm for 1 min was followed by slower stirring at 80 rpm for 30 min, and after that the system was left to sediment for 1 h. The same coagulation test was conducted with no coagulant (blank). After sedimentation for 1 h, residual turbidity was determined in 50 ml of upper clarified liquid, using turbidimeter and coagulation activity was calculated:

$$\text{Coagulation activity (\%)} = (M_b - M_s) \times 100 / M_b \quad [1]$$

were  $M_b$  and  $M_s$  are the turbidities of the blank and the sample, respectively.

## RESULTS AND DISCUSSION

Considering the fact that several different strains of bean were available as source for natural coagulants, the first step was to analyse them. Results of analyses of solid samples of beans are presented in Table 1.

As can be seen from these results, all of four bean strains have similar contents of dry matter. A little bit higher content of dry matter was determined in sample 2, and a little bit higher content of nitrogen in sample 4. Previous investigations showed that proteins exhibited coagulation activity (11), and this was the reason why we analysed the content of proteins. The protein content varied significantly between bean strains, and it could be said that sample 4 had by about 25 % higher content of proteins than sample 3. The fact that different strains of bean had different contents of proteins and other compounds was confirmed in previous investigations (12).

**Table 1.** Results of the analysis of solid bean samples

	Bean sample			
	1	2	3	4
Dry matter (%)	88.9	92.1	88.5	88.5
Content of nitrogen (% dm)	3.6	3.2	3.1	4.1
Content of proteins (% dm)	22.4	20.2	19.7	25.6

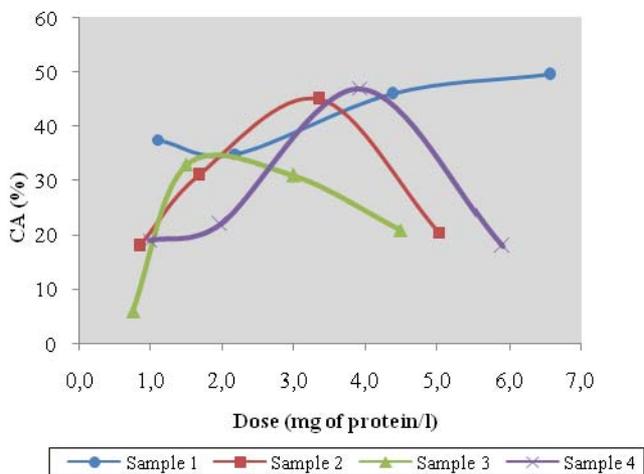
Table 2 gives the results of the analysis of extracts obtained from solid samples. The highest content of soluble nitrogen was found in sample 1. Also, the best extraction effi-

ciency (97.5 %) was obtained with sample 1. The amount of extracted proteins was not proportional to the content of proteins in solid samples. The efficiency of extraction determined for sample 4 was not so good, although this sample had the highest content of proteins.

**Table 2.** Results of the analysis of bean extracts

	Bean sample			
	1	2	3	4
Content of soluble nitrogen (mg/l)	350	268	239	315
Efficiency of extraction of nitrogen- containing compounds (%)	97.5	82.8	75.9	77.0

Figure 1 shows the influence of dose of different bean extracts on the coagulation activity. It can be seen that sample 2 and sample 4 showed very similar behavior, with maximum of coagulation activity at applied doses of 3.35 mg/l and 3.94 mg/l respectively. Sample 3 showed maximum of coagulation activity at a dose of 1.49 mg/l. In comparison with other samples, sample 1 had different behavior. This extract was efficient at lower, as well as at higher doses. It had maximum of coagulation activity at a dose of 6.56 mg/l, and with six times lower dose (1.09 mg/l) the coagulation activity decreased by 25 % in comparison with the maximum. Good coagulation activity was achieved for all of the samples when an optimal dose of the coagulant was applied. Similar results were also obtained with extracts of other Leguminose species (8), while natural coagulants obtained from *Moringa oleifera* seeds showed a little bit higher coagulation activity (6).



**Figure 1.** Effect of dose of different bean strains extracts on coagulation activity (CA)

Organic matter in water has a bad influence on water quality. Its presence in water can change color and clarity, and can lead to the appearance of specific smell and taste of water. Since natural coagulants are of organic nature, in the next step we wanted to investigate how they influence the content of organic matter after performing coagulation tests.

Content of organic matter in the water after performing coagulation tests was assessed by determining the permanganate demand. As first, jar tests with a dose of 3 ml/l of different coagulants were performed. After separating the upper clear parts, permanganate demand was determined in each of them. Simultaneously, permanganate demand was determined for the blank, and it was 10.6 mg KMnO<sub>4</sub>/l. The obtained results are shown in Table 3. Content of organic matter increased about two times in comparison with the blank. The reason for this were high doses of coagulants that were applied.

**Table 3.** Permanganate demand in water after performing coagulation tests

	Bean sample			
	1	2	3	4
Permanganate demand (mg KMnO <sub>4</sub> /l)	22.3	22.1	22.6	22.6

## CONCLUSION

Considering performed experiments and results obtained in the investigation of the coagulation activity of the extracts of natural coagulants obtained from various strains of bean, it is possible to derive the following conclusions:

- All investigated strains of bean showed potential to be used for preparing coagulants for water clarification.
- Turbidity of model water was decreased by 5 – 50 % by using natural coagulants obtained from different strains of bean.
- Samples 1, 2 and 4 showed maximum of coagulation activity (about 45%) in the range of applied doses of coagulants from 3.5 mg/l to 4.5 mg/l.
- Sample 3 showed a lower coagulation activity (maximum about 33%) in comparison with other samples, but at a significantly lower dose of coagulant – 1.5 mg/l.
- Content of organic matter in the water after coagulation tests performed with all samples was high, twice higher than it was in the blank.

## Acknowledgement

This research was supported by the grant number BTN 20009 from the Ministry of Science and Technological Development of the Republic of Serbia.

## REFERENCES

1. D.R. Crapper, S.S. Krishnan and A.J. Dalton: Brain Aluminium Distribution Alzheimer's Disease and Experimental Neurofibrillary Degeneration, *Science*, **180** (1973) 511-513.
2. C.N. Martyn, D.J.P. Barker, C. Osmond, E.C. Harris, J.A. Edwardson and R.F. Lacey: Geographical Relation between Alzheimer's Disease and Aluminium in Drinking Water, *The Lancet*, **1** (1989) 59-62.
3. R.G. Miller, F.C. Kopfler, K.C. Kelty, J.A. Stober and N.S. Ulmer: The Occurrence of Aluminium in Drinking Water, *J. AWWA*, **76** (1984) 94-101.
4. D.D. Mccollister, E. Oyen and V.K. Rowe: Toxicology of Acrylamide, *Toxicol. Appl. Pharmacol.*, **6** (1964) 172-181.
5. Sutherland J.: *M. oleifera* in water treatment, [www.le.ac.uk/engineering/staff/Sutherland/moringa/moringa.htm](http://www.le.ac.uk/engineering/staff/Sutherland/moringa/moringa.htm) (accessed 14 September 2010).
6. S.A. Muyibi and L.M. Evison: Optimizing Physical Parameters Affecting Coagulation of Turbid Water with *Moringa oleifera* Seeds, *Water Res.* **29**, 12 (1995) 2689-2695.
7. T. Ocuda, A.U. Beas, W. Nishiima and M. Okada: Improvement of Extraction Method of Coagulation Active Components with *Moringa oleifera* Seed, *Water Res.*, **33** (1999) 3373-3378.
8. M. Šćiban, M. Klačnja and J. Stojimirović: Investigation of Coagulation Activity of Natural Coagulants from Seeds of Different Leguminose Species, *Acta Periodica Technologica*, **36** (2005) 81-87.
9. MEBAK: Brautechnische Analysenmethoden, MEBAK, Freising-Weihenstephan (1997), Bd. I, p. 171.
10. Drinking water (in Serbian), Savezni zavod za zdravstvenu zaštitu, NIP "Privredni pregled", Beograd, 1990.
11. M. Antov, M. Šćiban and N. Petrović: Proteins from Common Bean (*Phaseolus vulgaris*) Seed as a Natural Coagulant for Potential Application in Water Turbidity Removal, *Bioresource Technology* **101**, 7 (2010) 2167-2172.
12. A. Tepić, B. Vujičić, M. Vasić and A. Lučić: Amino Acids and Phytic Acid in some Serbian Varieties of Dry Beans (*Phaseolus vulgaris*); 2<sup>nd</sup> International Congress on Food and Nutrition, Istanbul, 24-26 October 2007, Abstrac Book, p. 180.

## ИСПИТИВАЊЕ КОАГУЛАЦИОНЕ АКТИВНОСТИ ПРИРОДНИХ КОАГУЛАНАТА ЕКСТРАХОВАНИХ ИЗ РАЗЛИЧИТИХ СОРТИ ПАСУЉА

Марина Б. Шћибан, Мирјана А. Васић, Јелена М. Продановић, Мирјана Г. Анто, Миле Т. Клашња

Коагулација и флокулација представљају најчешће примењиване методе за бистрење воде, и углавном се изводе додатком хемијских коагуланата. У овом раду је испитивана коагулациона активност екстраката добијених из семена различитих сорти пасуља, а с циљем да се утврди могућност њихове примене као природних

коагуланата за уклањање мутноће воде, и да се утврди утицај сорте пасуља на коагулациону активност. Екстракција активних компоненти је изведена са 100 ml дестиловане воде из 1 g самлевоног узорка. У чврстим узорцима је одређен садржај суве материје и азота, а у екстрактима садржај растворљивог азота, на основу чега је одређена ефикасност екстракције једињења са азотом. За одређивање коагулационе активности екстраката изведен је цар-тест, додавањем различитих доза екстраката модел води, чији је рН 9 и почетна мутноћа 35 NTU. Након мешања у одређеном временском интервалу, чаше су остављене да се њихов садржај исталожи, а затим је у бистром делу одређивана мутноћа, и преко ње коагулациона активност. Такође је утврђен допринос сваког од екстраката порасту садржаја органских материја у води након њеног бистрења у односу на слепу пробу, а преко перманганатног броја. Овим експериментима потврђено је да се екстракти свих испитиваних сорти пасуља могу успешно применити као природни коагуланти.

Received 27 September 2010  
Accepted 8 November 2010