INFLUENCE OF THE COMPOSITION OF COMMON BEAN EXTRACTS ON THEIR COAGULATION ABILITY

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Coagulation and flocculation are the most used methods for removal of turbidity of water. Recently, many studies have focused on the investigation of natural coagulants for this purpose. In view of the fact that extracts of common bean have coagulation activity, this study is concerned with the chemical composition of these extracts and their influence on the coagulation activity. Extraction was conducted with distilled water, 0.5M NaCl and 1M NaCl and total sugars content, proteins, phytic acid and total phenolics content and their coagulation activity were determined in the obtained extracts. These experiments confirmed that an extraction time of 10 minutes is sufficient for the extraction of active coagulant components from common bean seeds and that water is satisfactorily efficient and most economical solvent.

KEY WORDS: water clarification, natural coagulants, common bean, extract composition

INTRODUCTION

More and more studies have been done recently in order to replace chemical agents, which are used for water clarification with natural coagulants, because of the negative effects of chemical coagulants on human health and the environment. Some studies reported that there was a potential link between residual aluminum in water after clarification with aluminum sulfate, or polyaluminum chloride, and Alzheimer’s disease (1-3). It was also reported that monomers of some synthetic organic polymers that are used as flocculants, such as acryl amide, have neurotoxicity and strong carcinogenic properties (4).

Apart from being safer for human health, natural coagulants are biodegradable, unlike chemical coagulants. They can be produced or extracted from microorganisms, animal or plant materials.

The possibility of using plant material as natural coagulants has been known for centuries (5). Different natural materials have been used in traditional water treatment in rural areas of developing countries. Some ancient texts confirm that the seeds of Nirmali

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tree (Strychnos potatorum) were used in India for water clarification (5). Ground seeds of Moringa oleifera were used for water clarification by women in rural areas of Sudan (5). Mesquite bean (Prosopis juliflora), Cactus latifaria (6), seeds of Cassia angustifolia (7) peanuts (Arachis) (8), various bean (Phaseolus) (8), seeds from Moringa oleifera (9-13), and many others have been recently tested as a source of natural coagulants. Seeds from Moringa oleifera showed the best results in water clarification.

Our previous studies were focused on the detection of plant species from our region that could be used as natural coagulants. It was found that various strains of Leguminose can be used for this purpose (14, 15). The selection of these materials was made based on the fact they contain 20 – 30% of proteins, which were reported as potential coagulant agents in natural coagulants (16, 17).

The aim of this study was to analyze the chemical composition of the extracts obtained from whole seeds of common bean (total sugars, proteins, phytic acid, total phenolics) in order to determine their influence on coagulation activity of these extracts. Also, different extragents were used in order to determine their influence on the quantity of extracted components.

**EXPERIMENTAL**

**Preparation of extracts**

The whole seeds of white common bean (Phaseolus vulgaris) were used for the extraction. Seeds were ground by laboratory mill and sieved through the sieve with pore size of 0.4 mm. An amount of 1 g of the smaller fraction was suspended in 100 ml of distilled water, 0.5M NaCl or 1M NaCl. The suspensions were stirred on a magnetic stirrer for 10 min or 3 h in order to extract the coagulation active component. After the extraction the suspensions were filtered through the filter paper Macherey-Nagel MN 651/120, and the obtained filtrates were kept in a refrigerator.

**Coagulation test**

The coagulation activity was assessed by jar test using synthetic turbid water (model water) which was prepared by adding 5 ml of 1% kaolin suspension into 1 l of tap water just before coagulation test. The initial turbidity of this model water was about 35 NTU. The pH of the model water was adjusted to pH 9 by adding 33% NaOH in accordance with previous investigations (14). The jar test was carried out by adding 0.5 ml of extracts per liter of model water. Systems were stirred at 200 rpm for 1 min, followed by stirring at 60 rpm for 30 min, and after that the suspensions were left to sediment for 1 h. The same coagulation test was conducted without the coagulant (blank). In 50 ml of upper clarified liquid the residual turbidity was determined. Coagulation activity was calculated by using the next formula:

\[
\text{Coagulation activity (\%) } = \left( \frac{M_b - M_s}{M_b} \right) \times 100
\]

where \(M_b\) and \(M_s\) are the turbidities of the blank and the sample, respectively.

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Analytical methods

The following parameters were determined in the extracts:
1. Total sugars were determined according to the valid Regulations (18).
2. Content of total nitrogen was determined by the Kjeldahl method (19). Protein content was calculated as total nitrogen content (mg/l) x 6.25.
3. Content of phytic acid was determined according to Haug and Lantzsch (20).
4. Total phenolics were determined using the Folin-Ciocalteu reagent. The reaction mixture was prepared by mixing 5 ml of extract, 75 ml of distilled water and 5 ml of Folin-Ciocalteu’s reagent. The subsequent steps were conducted according to the Folin-Ciocalteu method (21).
5. Water turbidity was measured by turbidimeter WTW Turb 550 IR and expressed in nephelometric turbidity units (NTU).
6. pH was measured using the pH-meter Oakton Ion 6.
7. Isoelectric point and particle size distribution of the extract were measured on a Zetasizer Nano ZS, Malvern Instruments, U.K.

Each analysis was carried out in triplicate and the results are presented as 95% confidence intervals of mean.

RESULTS AND DISCUSSION

Influence of the extraction time on the composition of extracts

The extraction of the ground seed was conducted with distilled water for 10 minutes and 3 hours in order to investigate the influence of the extraction time on the extraction efficiency. In our previous studies the extraction was conducted for 10 minutes (14), but the methodology of determination of individual components in plant materials required 3 hours of extraction. Extractions were repeated two times. Content of total sugars, proteins, phytic acid and total phenolic content were determined in all extracts. Coagulation tests were also performed. The results of these analyses are given in Table 1.

Table 1. Results of the analyses of the bean seed extracts obtained after 10 min and 3h extraction, and their coagulation activities

<table>
<thead>
<tr>
<th>Extraction time</th>
<th>Extract 1</th>
<th>Extract 2</th>
<th>Average</th>
<th>Total sugars content (mg/l)</th>
<th>Proteins (mg/l)</th>
<th>Phytic acid (mg/l)</th>
<th>Total phenolics content (mg/l)</th>
<th>Coagulation activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>725±28.4</td>
<td>668±54.8</td>
<td>697</td>
<td>1947</td>
<td>15.76±0.95</td>
<td>1.07±0.006</td>
<td>33.0±2.47</td>
<td>33.1±1.01</td>
</tr>
<tr>
<td>Average</td>
<td>725±28.4</td>
<td>755</td>
<td></td>
<td>1914</td>
<td>15.76±0.95</td>
<td>1.07±0.006</td>
<td>33.05</td>
<td>33.05</td>
</tr>
<tr>
<td>3 hours</td>
<td>811±11.7</td>
<td>699±37.3</td>
<td>755</td>
<td>1914</td>
<td>13.69±1.67</td>
<td>1.16±0.017</td>
<td>38.0±2.18</td>
<td>38.0±2.18</td>
</tr>
<tr>
<td>Average</td>
<td>811±11.7</td>
<td>755</td>
<td></td>
<td>1914</td>
<td>13.69±1.67</td>
<td>1.16±0.017</td>
<td>38.05</td>
<td>38.05</td>
</tr>
</tbody>
</table>

Increase (%) +8.32 +0.26 -15.89 +7.21
Bean extracts contain the most proteins, significantly less amount of total sugars, and very low concentrations of phytic acid and total phenolics. This is expected, and it is in accordance with the contents of these components in bean seeds. It was reported that the content of total sugars, proteins and phytic acid in common bean seeds was about 7.5%, 24% and 1%, respectively (22). Our investigations showed that 90% of total sugars content and 75% of proteins were extracted from seeds in water during the extraction. Phytic acid was extracted in an amount of just 10%.

The determination of total phenolics content was slightly more precise than the determination of total sugars and phytic acid. The reliability of all these methods was high since the variations of the mean value were small. Coefficients of variation for three replicated measurements in one sample for total phenolics varied from 0.5% to 1.2% (depending of sample), for total sugars from 0.5% to 2.7% and for phytic acid from 2% to 4%.

There was no big difference between the determined contents in two extracts, obtained under the same conditions (the same extraction time), except for phytic acid. It can be concluded that the determination of phytic acid by the applied method is not precise enough.

Extracts obtained in 3 hours extraction contain a slightly higher amount of total sugars and phenolic components compared to the extracts obtained in 10 minutes extraction. The phytic acid determination showed that the content of this component is significantly lower in the 3-h extract. Proteins content is almost the same in both extracts.

The results in Table 1 show that an extraction time of 10 minutes is sufficient for the extraction of active coagulant components from common bean seeds, because the quantities of determined components in both types of extracts were similar. Coagulation activity is slightly higher for the extract obtained in 3 hours extraction, but this does not justify extending of the time of extraction.

**Isoelectric point (IEP) and particle size distribution**

Isoelectric point of the 10 minute water extract is determined by measuring of zeta potential at different pH values of the extract (Figure 1).
The zeta potential was equal 0 at pH value of 3.61 therefore this pH value represents the isoelectric point. Hence some extract components are negatively charged at pH above IEP, it was concluded that the best conditions for coagulation test were higher pH values. This is in accordance with our previous investigations, when it was determined that water extracts of common bean had the highest coagulation activity at pH 9 (14).

**Figure 2.** Particle size distribution

The particle size distribution in water extract was also determined. Results are shown in Figure 2 as a number distribution. The X axis shows the distribution of size classes, while the Y axis shows the number of particles as a percent of total number. Diameters of all particles were in the range of 37 – 458 nm, but the most of the particles (19.2%) had a diameter of 68 nm.

**Influence of NaCl on composition of extracts**

Previous studies showed that the presence of NaCl in the solvent affected the extraction of coagulation agents (23). In order to determine this influence the extraction was conducted with 0.5M NaCl and 1M NaCl for 10 minutes, and compared with extraction with distilled water as a solvent. The obtained results are presented in Table 2.

**Table 2.** The influence of applied solvent on the extracts composition and their coagulation activities

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extract</th>
<th>Total sugars content (mg/l)</th>
<th>Proteins (mg/l)</th>
<th>Phytic acid (mg/l)</th>
<th>Total phenolics content (mg/l)</th>
<th>Coagulation activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Extract 1</td>
<td>725±28.4</td>
<td>1947</td>
<td>13.76±0.95</td>
<td>1.07±0.006</td>
<td>33.0±2.47</td>
</tr>
<tr>
<td></td>
<td>Extract 2</td>
<td>668±54.8</td>
<td>1870</td>
<td>15.19±0.92</td>
<td>1.15±0.011</td>
<td>33.1±1.01</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>697</td>
<td>1909</td>
<td>14.47</td>
<td></td>
<td>33.05</td>
</tr>
<tr>
<td>0.5 M NaCl</td>
<td>Extract 1</td>
<td>602±26.5</td>
<td>1728</td>
<td>16.61±0.17</td>
<td>1.05±0.008</td>
<td>19.4±2.18</td>
</tr>
<tr>
<td></td>
<td>Extract 2</td>
<td>505±18.4</td>
<td>1991</td>
<td>13.82±0.15</td>
<td>1.27±0.017</td>
<td>21.2±0.91</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>553.5</td>
<td>1860</td>
<td>15.21</td>
<td>1.16</td>
<td>20.3</td>
</tr>
<tr>
<td>1 M NaCl</td>
<td>Extract 1</td>
<td>384±27.5</td>
<td>2012</td>
<td>10.95±0.12</td>
<td>1.17±0.008</td>
<td>29.8±1.42</td>
</tr>
<tr>
<td></td>
<td>Extract 2</td>
<td>428±19.2</td>
<td>2056</td>
<td>12.53±1.23</td>
<td>1.24±0.022</td>
<td>31.6±1.43</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>406</td>
<td>2034</td>
<td>11.74</td>
<td>1.20</td>
<td>30.7</td>
</tr>
</tbody>
</table>
The extracts obtained by extraction with NaCl solutions contained slightly higher amounts of phenolic components compared to the water extract. Total sugars content was the highest in the water extract and the lowest in the extract obtained with 1 M NaCl, while for the proteins and phytic acid there could not be established a logical correlation. It was found that the water extract had the highest coagulation activity (33.05%), followed by the extract obtained with 1 M NaCl (30.7%). The coagulation activity of the extract obtained with 0.5 M NaCl was significantly lower than the other ones. This means that the presence of NaCl in the solvents influenced the composition of the extracts, but did not improve their coagulation properties, and it was recommended that the extraction should be performed with distilled water. Adamović (23) and Ćuvardić (24) have reported that, when the common bean and acorn have been extracted with water, 0.5M or 1M NaCl, the extracts with 0.5M NaCl had slightly higher coagulation activity, and in opposite, it was found that the water extract of chestnut was the best (25). Based on these findings it can be concluded that water is satisfactorily efficient and most economical solvent, and that addition of NaCl is not necessary.

CONCLUSION

The obtained results can not provide an explanation of the correlation between extract composition and coagulation activity of the investigated extracts. The reason for this is probably the fact that the extracts contain a lot of different components since they are crude.

The results showed that an extraction time of 10 minutes is sufficient for extraction of active coagulant components from common bean seeds. Coagulation activity is slightly higher for the extract obtained in 3 hours extraction, but this does not justify the extension of extraction time.

In the experiments with the extragents of different NaCl concentration, it was found that the presence of NaCl in the extragents influenced differently the composition of the obtained extracts. The water extract had similar coagulation activity as the extract obtained with 1 M NaCl. The coagulation activity of the extract obtained with 0.5 M NaCl was significantly lower. This means that the presence of NaCl in the extragents did not improve coagulation properties of the extracts, and it can be concluded that water is satisfactorily efficient and most economical solvent.

Acknowledgement

This research was supported by the Ministry of Education and Science of the Republic of Serbia as a part of the Project Number III 43005.

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селине и укупних фенола и одређена је њихова коагулациона активност. Након обраде резултата ових анализа, није установљен јасан утицај појединих компонената екстраката на њихову коагулациону активност. Утврђено је да је довољно време за екстракцију активних компоненти 10 минута, и да је вода најбољи екстракгенс у том погледу.

Кључне речи: бистрење воде, природни когуланти, бели пасуљ, састав екстракта

Received 30 June 2011
Accepted 08 August 2011