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Downstream process engineering evaluation of transgenic soybean seeds as host for recombinant protein production

Goran Robić [a], Cristiane S. Farinas [a], Elíbio L. Rech [b], Sônia M.A. Bueno [a], Everson A. Miranda [a,*]

[a] *Departamento de Processos Biotecnológicos, Faculdade de Engenharia Química, Universidade Estadual de Campinas, CP 6066, CEP 13083-970 Campinas, SP, Brazil*

[b] *Laboratório de Transferência de Genes, EMBRAPA Recursos Genéticos e Biotecnologia, CP 02372, CEP 70770-900 Brasília, DF, Brazil*

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Abstract

The advantages of using seeds for the production of recombinant proteins with plant-based expression system has been demonstrated by several researchers. The high productivity makes soybean a potential system for large-scale recombinant protein production. However, there is a lack of detailed engineering studies of the downstream process (DSP) of recombinant proteins produced in transgenic soybean. In this work, we evaluated the use of transgenic soybean seeds as hosts for the production of recombinant proteins from a downstream process (DSP) engineering standpoint. Recombinant β-glucuronidase (rGUS), was used as a model for extraction and purification studies. This study showed, that even a protein with acidic p*I* (rGUS) can be successfully separated from native soybean proteins, which also have acidic p*I*. Maximum GUS specific activity (9.5×10^3 U/mg) with high total activity recovery (8.9×10^4 U/mL) was obtained using a simple extraction solution composed of 50 mmol/L citrate buffer at pH 5.25. Purification of rGUS was evaluated by a two-step chromatographic procedure – anion-exchange followed by hydrophobic interaction chromatography – which was compared to the purification of rGUS from transgenic corn and canola. Overall purification factor and activity recovery obtained were 97.3 and 110% (a value higher than 100% probably due to removal of an inhibitor). Comparison of this study with similar ones made with corn and canola seeds indicates that in terms of DSP soybean seeds can be considered a potentially viable plant system for the production of recombinant proteins.

Keywords: Soybean seeds; Extraction; Transgenic plants; Protein purification; Glucuronidase

1. Introduction

A large variety of plant species are being evaluated as host for the production of recombinant proteins with industrial and pharmaceutical applications. The spectrum of plants ranges from cereal crops such as maize, rice, barley, and wheat to legumes like pea and soybean, and also the leafy crops alfalfa and tobacco. According to Stoger et al. [1], seed-based hosts can offer several advantages for the production of recombinant proteins, since the accumulation of proteins takes place in a relatively small volume and stable environment in which they are protected from degradation. Although soybean is considered a potential seed-based bioreactor there are only few reports addressing this issue. In the work of Zeitlin et al. [2], an antibody against herpes simplex virus was expressed constitutively in the plant. The plants were harvested and pressed to extract the recombinant protein that was purified by affinity chromatography. Philip et al. [3] reported the expression of casein in soybean seeds, which was purified also by affinity chromatography. Recently, the expression of the human growth hormone in transgenic soybean was reported by Russel et al. [4]. The reason for relatively few reports on this subject is that a high level expression of a recombinant protein has not been achieved yet in soybean seed. As pointed out by Twyman et al. [5], recombinant protein expression higher than 0.1% of the total soluble protein in seeds is needed for a plant system to be competitive with other expression systems. However, soybean seeds offer the advantages of having a low production cost, and from a regulatory standpoint, a reduced risk of contamination by pollen, since soybean is largely self-pollinating. Therefore, it is a system that needs to be studied in order to have a fully evaluation of its potential.

* Corresponding author. Tel.: +55 19 3521 39 18; fax: +55 19 3521 3890.
E-mail address: everson@feq.unicamp.br (E.A. Miranda).

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Besides the right choice of plant specie, the success of the large-scale use of plants as bioreactors depends also on the downstream processing (DSP) necessary for purification of the recombinant protein. Extraction is a key step in an efficient DSP, since it defines the characteristics of the solution from which the recombinant protein will be purified. Plant species contain different levels of compounds (e.g., native proteins, soluble carbohydrates, phenolic compounds, and lipids) that can be cosolubilized with the recombinant product during the extraction, which are deleterious to the efficiency of the process, separation media, and equipment. Therefore, an efficient extraction condition that minimizes the coextraction of the plant native compounds could simplify DSP operations.

According to Jefferson et al. [6] GUS is a homotetrameric enzyme (monomer molecular mass and p*I* of approximately 68 kDa and 5.5) that cleaves the β-linked terminal glucuronic acids in mono and oligosaccharides and phenols. Although one of the natural sources of GUS is *Escherichia coli*, a recombinant GUS produced in transgenic corn is currently commercialized [7,8]. It is widely used as a visual marker for analysis of gene expression in transgenic plant research. One important application of the expression of GUS is its use as a model protein in studies of process engineering strategies regarding DSP of recombinant proteins produced in transgenic plants [7–11].

Kusnadi et al. [7,8] described the production, purification, and characterization of GUS from transgenic corn seed. An estimated value of 70% of the GUS was extracted with 50 mmol/L sodium phosphate buffer at pH 7.5. The addition of 1% SDS and 2% β-mercaptoethanol resulted in complete extraction of GUS; however, the SDS caused apparent irreversible inactivation of the enzyme. In both reports rGUS was purified with anion-exchange, hydrophobic interaction, and size-exclusion chromatographies. A similar GUS extraction procedure was used by Zhang and Glatz [9] and Zhang et al. [10] with canola as the expression host. Canola extract was obtained by mixing 50 mmol/L sodium phosphate buffer at pH 7.0 with deffated meal at a 1:10 solid-to-liquid ratio for 30 min. Purification of GUS and three other polyaspartate fusions to GUS were studied using anion-exchange chromatography with different elution profiles. Bai and Glatz [11] studied the use of packed and expanded bed ion-exchange adsorption for rGUS purification from transgenic canola. They reported rGUS recovery of 112% and purification factor of 31 using a packed bed of Streamline-DEAE.

In this study we evaluated soybean as a bioreactor by studying the DSP – extraction and purification steps – of a model recombinant protein rGUS produced in the seeds of transgenic plants and comparing the results with reported studies with corn [7,8] and canola seeds [11]. The chromatographic steps (anion-exchange and hydrophobic interaction) were evaluated in terms of purification based on GUS specific activity, as done in the case of corn [7,8] and canola [11]. Aqueous extraction of rGUS, native proteins, carbohydrates, and phenolic compounds was evaluated as a function of pH of extraction solution. The reason to studied extraction of other compounds than rGUS is because native proteins are the major impurities in the extracts, phenolics are known to interfere with DSP since they may cause resin fouling, protein denaturation and degradation, and carbohydrates promote bacterial growth.

2. Materials and methods

2.1. Materials

Transgenic soybean seeds (cultivar BR-16, lines 8–19) expressing the GUS gene were provided by EMBRAPA, Brazil [12]. Genetic transformation was done using the particle bombardment technique, using a constitutive promoter. GUS substrate 4-methylumbelliferyl glucuronide (MUG) and 4-methylumbelliferone (MU) were from Sigma (USA). High-purity water prepared with a Milli-Q System (Millipore, USA) was used in all experiments. All other chemicals used were of at least analytical grade. A DU 650 spectrophotometer (Beckman, USA) and a F-4500 fluorescence spectrophotometer (Hitachi, Japan) were used for the spectrophotometric measurements.

2.2. Methods

2.2.1. Preparation of soybean flour

Soybean seeds were ground in a household coffee grinder by intermittent runs in order to avoid excessive heating (the temperature did not exceed 60 °C). The particles were separated using a set of sieves, resulting in flour with particles smaller than 0.5 mm. This flour was defatted with hexane at 60 °C for 6 h in Soxhlet-type equipment and stored at room temperature until its use in the extraction experiments.

2.2.2. Extraction protocol

In each run, 5 g of soybean flour were mixed with 100 mL of the 50 mmol/L sodium citrate buffer with appropriate pH (1:20 solid-to-liquid ratio) at room temperature for 30 min. Extraction was carried out in a 5.5 cm diameter 250 mL beaker using a mechanical stirrer (Q-251D, IKA Labortechnik, Germany) equipped with an axial-flow impeller (pitched-blade turbine with four blades, 4.0 cm in diameter at 45° angle and positioned 1 cm from the bottom) at a stirring rate of 500 rpm. After 30 min of mixing, each suspension was centrifuged at 15,000 × *g* for 20 min at 5 °C and polished by filtration through a 3 μm filter paper. Extractions for chromatographic experiments were done with 50 mmol/L citrate buffer pH 5.25.

2.2.3. Chromatographic procedures

Chromatographic experiments were carried out with either a Breeze HPLC Waters System (Waters, USA) or a system consisting of a Miniplus 3 peristaltic pump (Gilson, France), an Econo UV Monitor detector, and a fraction collector (both by Bio-Rad, USA) at a constant flow rate of 1.0 mL/min. The purification factor was calculated as the ratio between the specific GUS activity of the pool of eluted fractions and the specific GUS activity of the sample injected.

2.2.3.1. Anion-exchange chromatography. A volume of 5.5 mL of the anion-exchange resin DEAE-Sephadex A25 (Pharmacia, Sweden) was packed into a C10/10 glass column (10.0 cm ×

1.0 cm) (Amersham Biosciences, Sweden). The column was equilibrated with 50 mmol/L sodium phosphate buffer pH 7.00 (buffer A) and fed with different volumes of soybean seeds extracts (1.0, 2.0, 3.0, 4.0, and 5.0 mL). Feed samples were prepared with 50 mM sodium citrate buffer pH 5.25 as the extraction solvent and final pH was adjusted with 500 mM sodium phosphate pH 11.40, yielding a final feed solution with 50 mM sodium phosphate pH 7.00. After each feed injection, the column was washed with at least 55.0 mL of buffer A. Two elution procedures were tested: a linear gradient from 0 to 500 mmol/L of NaCl in buffer A (elution #1) and a step with 300 mmol/L NaCl in buffer A (buffer B) (elution #2). After each chromatographic run, the column was regenerated with a solution of 1.0 mol/L NaCl. Fractions were collected and analyzed for total protein and GUS activity.

2.2.3.2. Hydrophobic interaction chromatography. Fractions of elution #2 from anion-exchange chromatography containing GUS activity were combined as a pool and diluted 1:1 with a solution of 3 mol/L $(NH_4)_2SO_4$ in buffer B. A volume of 10.0 mL of this solution was then injected into a C10/20 glass column (20.0 cm × 1.0 cm) (Amersham Biosciences, Sweden) packed with 12.0 mL of Streamline-Phenyl gel (Pharmacia, Sweden). After feed injection, a washing step with 40.0 mL of 1.5 mol/L $(NH_4)_2SO_4$ in buffer B was carried out. Weakly adsorbed proteins were eluted using 0.5 mol/L $(NH_4)_2SO_4$ in buffer B followed by buffer B. rGUS was eluted with high-purity water. The column was regenerated using at least 250 mL of high-purity water. Fractions were collected and analyzed for total protein and GUS activity.

2.2.4. Analytical methods

GUS activity was quantified with a fluorometric assay based on the method of Jefferson [13] using the substrate MUG. One unit of GUS activity (U) will release 1 nmol of MU from MUG per minute at pH 7.0 and 37 °C. Total soluble protein (TSP) concentration in the extracts was determined with the method of Bradford [14] using bovine serum albumin (Sigma, USA) as a standard. Specific activity of GUS (U/mg) of a sample is defined as its total GUS activity (U) per total soluble protein mass (mg). SDS-PAGE (15% acrylamide gels) under nonreducing conditions were carried out with a Protean II System (Bio-Rad, USA) [15]. The gels were stained with Coomassie Blue or silver nitrate [16] and a standard protein mixture (M3913 Sigma, USA) was used as a molecular mass protein marker. Phenolics were quantified using D-catechin (Sigma, USA) as standard [17]. Soluble carbohydrates were quantified as total sugars (TS) using the dinitrosalicylic acid (Synth, Brazil) [18]. Glucose (Synth, Brazil) was used as standard for determination of TS.

3. Results and discussion

3.1. Extraction studies

The rGUS extraction kinetics was relatively fast, since the maximum concentration was achieved in 25 min using either 50 mmol/L sodium citrate or phosphate buffer—pH 4.0–7.6

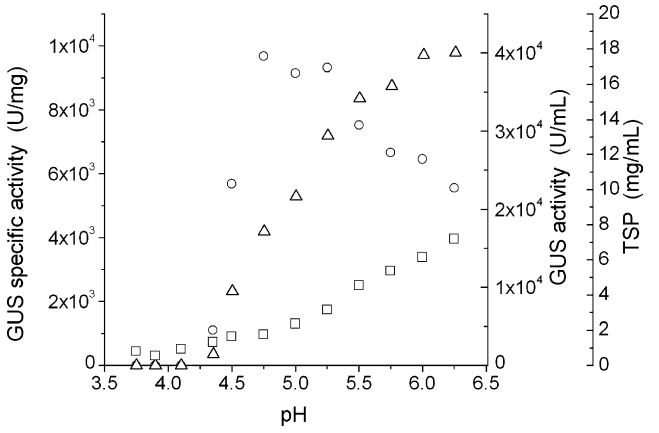

Fig. 1. GUS activity and specific activity and total soluble protein (TSP) in the aqueous extracts of transgenic soybean seeds using 50 mmol/L sodium citrate buffer. (◯) GUS specific activity, (△) GUS activity and (□) total soluble protein.

(data not shown). Therefore, a 30-min period of extraction was selected for the experiments that followed. This extraction time is in the same order of magnitude of the time reported for rGUS extraction from corn and canola seeds: Kusnadi et al. [7] studied the extraction of rGUS from transgenic corn using 50 mmol/L sodium phosphate buffer at neutral pH and considered 15 min sufficient to extract rGUS, whereas Zhang et al. [10] took 30 min for the extraction of rGUS from transgenic canola using the same buffer. This means that 30 min is enough to extract proteins not bound to insoluble components of the seed of these three plants.

The effect of pH on rGUS extraction was investigated for the pH range of 3.75–6.25 in the absence of NaCl in the extraction buffer. Preliminary tests showed that the presence of NaCl (30–300 mmol/L) in the extraction buffer (in the range of pH 4.0–7.6) raised concentration of TSP and lowered GUS activity of the extract (data not shown). Both GUS activity and TSP concentration increased with pH (Fig. 1). However, GUS specific activity showed a maximum value in the pH range of 4.75–5.25 (approximately 9.5×10^3 U/mg). Therefore, 5.25 was considered the optimum pH for rGUS extraction, since the GUS activity yield was the highest in this pH range (2.9×10^4 U/mL). Based on these results, 50 mmol/L citrate buffer at pH 5.25 was selected as the extraction solvent in the purification studies.

The protein molecular mass profiles of the extracts were obtained through SDS-PAGE. A behavior similar to the one obtained by Wolf et al. [19] was observed. Those authors noted that when the pH of the extraction suspension was near 7.0, β-conglycinin (subunit molecular masses of approximately 48, 63, and 76 kDa), glycinin (subunit molecular masses of approximately 22, 34, and 40 kDa), and soybean trypsin inhibitor (molecular mass of 20 kDa) were the major proteic components of the extract; at pH around 4.0 (near the p*I* of soybean proteins,) the major proteic components were lipoxygenase (molecular mass of 100 kDa), β-amylase (molecular mass of 55 kDa), agglutinin (molecular mass of 32 kDa), and soybean trypsin inhibitor. In this work extractions at pH 4.35 and 4.75 resulted in extracts composed basically of proteins with low molecular mass (smaller than 100 kDa) and therefore poor in two major soybean seed proteins: glycinin and β-conglycinin (Fig. 2). However,

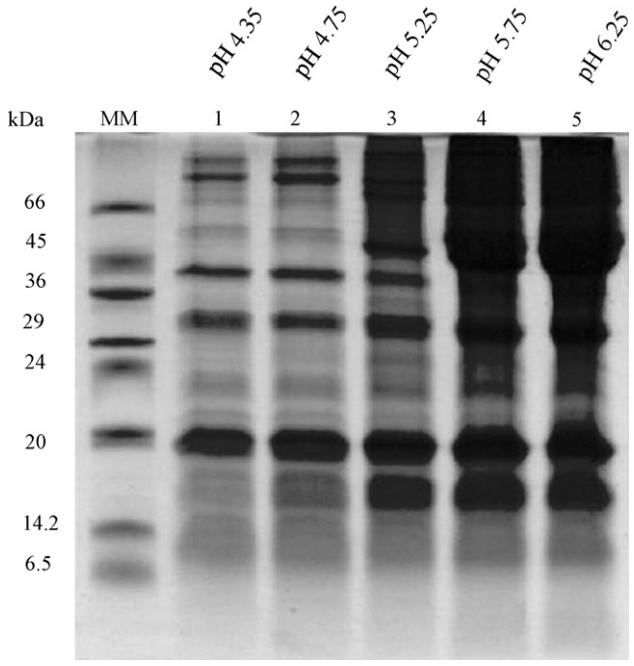

Fig. 2. SDS-PAGE analysis under nonreducing conditions (15% acrylamide, Coomassie stained) of transgenic soybean extracts prepared with 50 mmol/L sodium citrate buffer at different pH values; 10 μL of extract per lane. MM: molecular mass markers. Lane 1: pH 4.35; lane 2: pH 4.75; lane 3: pH 5.25; lane 4: pH 5.75; lane 5: pH 6.25.

extracts with pH higher than 4.75 had a richer composition in proteins of higher molecular masses.

The concentration of TS and phenolics was practically constant for all extractions, with average 8.0 ± 0.5 mg/mL for TS and 0.47 ± 0.08 mmol/L for phenolics. These values are of the same magnitude as the ones described for the extracts from endosperm of transgenic corn [20]. Since the activity of rGUS remained unchanged during 1 week at 5 °C the concentration of phenolic was considered to be lower than the concentration needed to cause the rGUS precipitation [21].

3.2. Purification studies

The studies of rGUS purification were carried out with anion-exchange followed by hydrophobic interaction chromatographies. The results obtained here were compared with results of similar studies done with extracts of corn [7,8] and canola [11]. Thus, the purification strategies employed here were similar to the ones described by the cited authors. Adsorption buffer and resins were the same and the elution was investigated.

3.2.1. Anion-exchange chromatography

Two elution strategies were evaluated (in both cases feed volume was 5.0 mL) and the first one was a linear gradient of NaCl from 0 to 500 mmol/L (elution #1). Most of the soybean proteins did not bind to the anion-exchange resin and were eluted in the flow through (Fig. 3), as in the case of canola proteins [10]. Weakly bound proteins were eluted in a peak at 70 min (100 mmol/L of NaCl). The chromatogram had two other peaks for concentrations of NaCl of 295 and 365 mmol/L that were

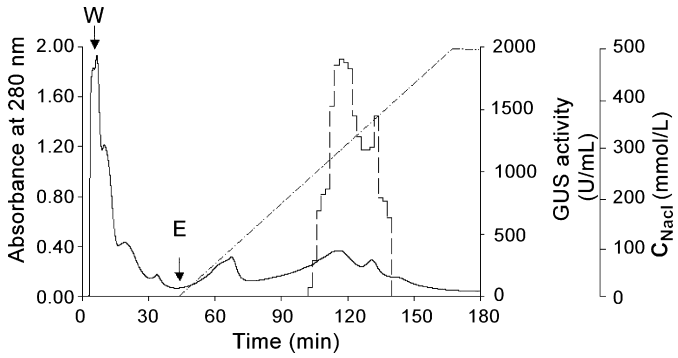

Fig. 3. Anion-exchange chromatogram of transgenic soybean extract with elution #1. Elution was carried out with a linear gradient of NaCl concentration from 0 to 500 mmol/L in the adsorption buffer (50 mmol/L phosphate buffer pH 7.00) at a rate of 4 mmol/L NaCl per minute. Arrows show the beginning of washing (W) and elution (E) steps. Absorbance at 280 nm (—); GUS activity (- - -); C_{NaCl} (·····).

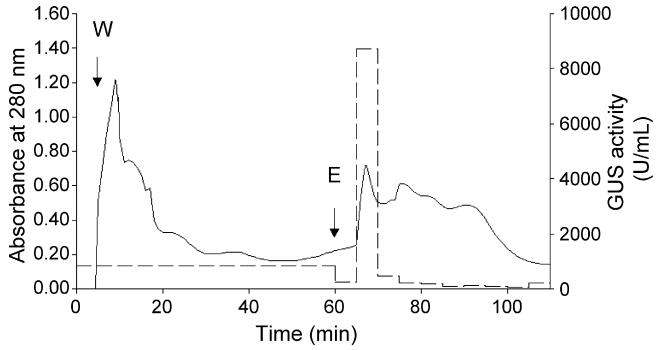

Fig. 4. Anion-exchange chromatogram of transgenic soybean extract with elution #2. Elution was carried out with an one-step gradient of 300 mmol/L NaCl in the adsorption buffer (50 mmol/L phosphate buffer pH 7.00). Arrows show the beginning of washing (W) and elution (E) steps. Absorbance at 280 nm (—); GUS activity (- - -).

combined since they showed the presence of GUS activity. The second elution strategy tested (elution #2) was carried out with a single step of 300 mmol/L NaCl (Fig. 4). rGUS was recovered in the fractions collected between 65 and 70 min. rGUS purification factor and activity yield obtained with the two elution strategies are summarized in Table 1. The best elution strategy was elution #2, given that it allowed for the higher purification factor (3.6).

Since more than 50% of GUS activity was lost (mostly in the flow through), different feed volumes (1.0, 2.0, 3.0, and 4.0 mL)

Table 1
Purification of rGUS from transgenic soybean seed extract by anion-exchange chromatography

Elution strategy	Feed volume (mL)	C_{NaCl}[a] (mmol/L)	Purification factor	Activity recovery (%)
Elution #1	5.0	0–500	2.3	43[b]
Elution #2	5.0	300	3.6	41[b]
Elution #2	4.0	300	6.2	63[c]

Adsorption buffer: 50 mmol/L sodium phosphate pH 7.00, column bed volume 5.5 mL.

[a] C_{NaCl}: NaCl concentration in mmol/L.

[b] GUS initial activity of 10^6 U/mL corresponds to 100%.

[c] GUS initial activity of 0.8×10^6 U/mL corresponds to 100%.

were tested, using the elution #2, aiming to achieve a higher recovery of rGUS. The best result was obtained with 4.0 mL of feed volume, resulting in a purification factor of 6.2 and a 63% activity recovery (Table 1). Even though lower volumes resulted in a similar yield, the purification factors were lower (data not shown).

Purification factors for rGUS from corn kernel extracts after an anion-exchange chromatography were 5 and 8 [7,8]. These values are of the same order of magnitude as the purification factors obtained in this study. Nevertheless, purification factors obtained for transgenic canola [11] were as high as 32. The difference between these results is due to the fact that the majority of native canola proteins have a basic p*I*. Consequently, when using anion-exchange chromatography, the native canola proteins are easily separated from recombinant proteins that have an acidic p*I*, which is the case of rGUS (p*I* of 5.5). In accordance to the predictions of Menkhaus et al. [22], a favorable scenario for the use of soybean seeds as bioreactor is the one in which the target protein is larger than 100 kDa and its p*I* is higher than 7.

Proteins that coeluted with rGUS were mainly β-conglycinin, lipoxygenase, and soybean trypsin inhibitor (Fig. 5). The coelution of these proteins could be explained by their p*I* (in the range of 4.00–5.65) close to the p*I* of rGUS. The fact that glycinin did not coeluted with rGUS could be due to the fact that glycinin is composed of both acidic and basic subunits [23] and therefore, it showed a weaker adsorption onto the DEAE resin.

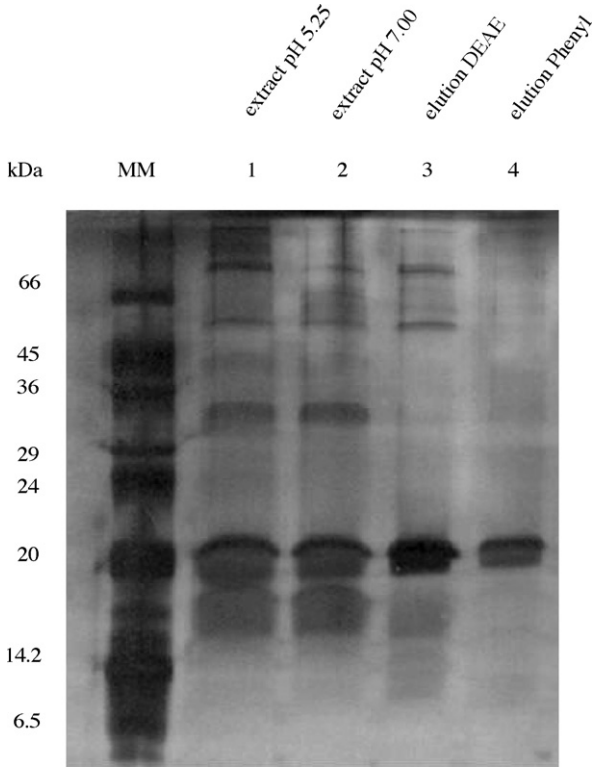

Fig. 5. SDS-PAGE analysis under nonreducing conditions (15% acrylamide, silver stained according to Morrisey [16]) of rGUS purification from soybean seeds; 0.1 μg of total protein per lane. MM: molecular mass marker. Lane 1: soybean extract at pH 5.25. Lane 2: soybean extract with pH adjusted to 7.00. Lane 3: elution with 300 mmol/L of NaCl in 50 mmmol/L phosphate buffer pH 7.00 (elution #2) using DEAE-Sephadex column. Lane 4: elution with water Milli-Q using Streamline-Phenyl column. The low concentration of rGUS in the extracts (lower than 0.1%) did not allow the visualization of this protein band.

3.2.2. Hydrophobic interaction chromatography

Hydrophobic interaction chromatography (HIC) was employed as a second step for rGUS purification. The feed solution was the pool of fractions of the ion-exchange chromatography with 4.0 mL of extract as feed and desorption according strategy elution #2. The elution protocol for HIC employed was a three-step gradient with 500 mmol/L of $(NH_4)_2SO_4$ in buffer B, buffer B, and water (Fig. 6). Purification factor and activity for fraction collected between 180 and 195 min were 15.7 and 174%. The activity recovery higher than 100% was probably due to the removal of an unidentified inhibitor. The same phenomenon was described for the purification of rGUS from transgenic corn [7]. The authors also observed that commercial *E. coli* GUS added to extracts of corn and soybean seeds lost as much as 40% of the initial activity.

A total activity recovery of 230% was achieved for this chromatographic step. In order to compare the purification factor obtained for this step (15.7) with that of rGUS purification from corn kernel extracts (purification factor of 8 [7,8]), we divided the purification factor obtained in this work by 2.3 (to account for the activity reestablished after removal of the inhibitor), resulting in almost the same value (7). An attempt to evaluate the purity of rGUS by SDS-PAGE analysis showed that rGUS concentrations were below the detectable level, since no band was observed around the expected molecular mass for the rGUS monomer (68 kDa), under both reducing (data not shown) and nonreducing conditions (Fig. 5). Nevertheless, there was only a strong band around the molecular mass of 20 kDa, which probably corresponds to the soybean trypsin inhibitor. We presume that one more chromatographic step – size exclusion – would result in highly pure rGUS. The difference in molecular mass of rGUS and trypsin inhibitor is large enough to allow efficient separation (272 and 20 kDa). The DSP parameters purification factors and activity recoveries for the two chromatographic steps are

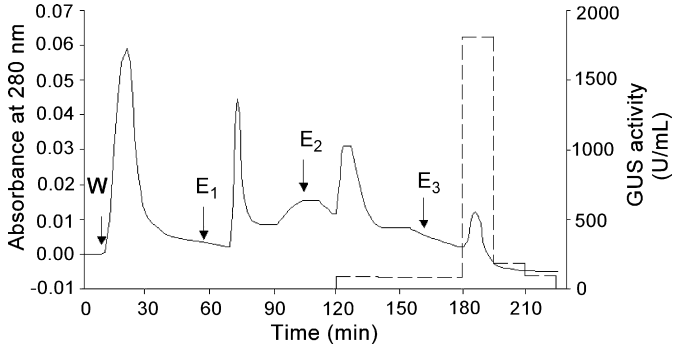

Fig. 6. Hydrophobic interaction chromatogram of transgenic soybean extract monitored by absorbance at 280 nm and rGUS activity. Elution was carried out with a three step gradient: 300 mmol/L NaCl and 0.5 mol/L $(NH_4)_2SO_4$ in the adsorption buffer (50 mmol/L phosphate buffer pH 7.0) (E_1), 300 mmol/L NaCl in the adsorption buffer (E_2) and high purity water (E_3). Arrows shows the beginning of washing (W) and elution (E_1, E_2, and E_3) steps. Absorbance at 280 nm (—); GUS activity (- - -).

Table 2
Purification of rGUS from soybean seeds in comparison with that from corn and canola seeds

Purification step	Soybean seed		Corn kernel [7]		Corn kernel [8]		Canola seed [11]	
	PF[a]	*R* (%)[b]	PF	*R* (%)	PF	*R* (%)	PF	*R* (%)
Extraction	1	100	1	100	1	100	1	100
AEC[c] (DEAE)	6.2	63	5	110	8	126	32	112
HIC[d] (phenyl)	97.3	110	40	46	62	81		

[a] PF: purification factor.
[b] *R*: rGUS activity recovery.
[c] AEC: anion-exchange chromatography.
[d] HIC: hydrophobic interaction chromatography.

presented in Table 2 together with related literature data. The overall parameters of this work are higher than for a similar work done with corn. However the anion-exchange parameters obtained with canola are superior than the ones obtained in this work.

4. Conclusions

This work showed that the optimum condition to extract rGUS from transgenic soybean seeds with minimum coextraction of native proteins is to use a low pH solution in the absence of NaCl. Under this condition, most of the native proteins extracted are low molecular mass proteins (lower than 66 kDa). Maximum rGUS specific activity was found using 50 mmol/L citrate buffer at pH 5.25. Purification studies lead to an overall yield of 110% and a purification factor of 97.3 using a two-step chromatographic procedure. On the other hand this study showed that if the recombinant protein has an acidic p*I* it still can be purified using only few chromatographic steps as long as it is has a hydrophobic surface, as in the case of GUS. Therefore, an ideal target recombinant protein to be produced in soybean seeds would have molecular mass higher than 100 kDa and a basic p*I* and/or a hydrophobic surface. Finally, evaluation of soybean in terms of the main DSP steps confirmed its potential as a commercial bioreactor for the production of recombinant proteins. Simple extraction buffer and values for purification parameters better than those reported for system currently used as host for commercial recombinant protein – corn – were obtained.

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References

[1] E. Stoger, J.K.C. Ma, R. Fischer, P. Christou, Sowing the seeds of success: pharmaceutical proteins from plants, Curr. Opin. Biotechnol. 16 (2005) 167–173.
[2] L. Zeitlin, S.S. Olmsted, T.R. Moench, M.S. Co, B.J. Martinell, V.M. Paradkar, D.R. Russell, C. Queen, R.A. Cone, K.J. Whaley, A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes, Nat. Biotechnol. 16 (1998) 1361–1364.
[3] R. Philip, D.W. Darnowski, P.J. Maughan, L.O. Vodkin, Processing and localization of bovine β-casein expressed in transgenic soybean seeds under control of a soybean lectin expression cassette, Plant Sci. 161 (2001) 323–335.
[4] D.A. Russell, L.A. Spatola, T. Dian, V.M. Paradkar, D.R. Dufield, J.A. Carrol, M.R. Schlitter, Host limits to accurate human growth hormone production in multiple plant systems, Biotechnol. Bioeng. 89 (2005) 775–782.
[5] R.M. Twyman, E. Stoger, S. Schillberg, P. Christou, R. Fischer, Molecular farming in plants: host systems and expression technology, Trends Biotechnol. 21 (2003) 570–578.
[6] R.A. Jefferson, S.M. Burgess, D. Hirsh, β-Glucuronidase from *E. coli* as a gene-fusion marker, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 8447–8451.
[7] A.R. Kusnadi, R.L. Evangelista, E.E. Hood, J.A. Howard, Z.L. Nikolov, Processing of transgenic corn seed and its effect on the recovery of recombinant β-glucuronidase, Biotechnol. Bioeng. 60 (1998) 44–52.
[8] A.R. Kusnadi, E.E. Hood, D.R. Wicher, J.A. Howard, Z.L. Nikolov, Production and purification of two recombinant proteins from transgenic corn, Biotechnol. Prog. 14 (1998) 149–155.
[9] C. Zhang, C.E. Glatz, Process engineering strategy for recombinant protein recovery from canola by cation exchange chromatography, Biotechnol. Prog. 15 (1999) 12–18.
[10] C. Zhang, R.T. Love, J.M. Jilka, C.E. Glatz, Genetic engineering strategies for purification of recombinant proteins from canola by anion chromatography: an example of β-glucuronidase, Biotechnol. Prog. 17 (2001) 161–167.
[11] Y. Bai, C.E. Glatz, Capture of recombinant protein from unclarified canola extract using streamline expanded bed anion exchange, Biotechnol. Bioeng. 81 (2003) 855–864.
[12] F.J.L. Aragão, L. Sarokin, G.R. Vianna, E.L. Rech, Selection of transgenic meristematic cells utilizing a herbicidal molecule results in the recovery of fertile transgenic soybean [/glycine max/(l.) merril] at a high frequency, Theor. Appl. Genet. 101 (2000) 1–106.
[13] R.A. Jefferson, K.J. Wilson, The GUS gene fusion system, in: S.B. Gelvin, R.A. Schilperoot, D.P.S. Verma (Eds.), Plant Molecular Biology Manual, vol. 1, B14, Kluwer Academic Publishers, Dortrecht, 1991, pp. 1–33.
[14] M.M. Bradford, A rapid and sensitive method for a quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[15] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of the bacteriophage T4, Nature 227 (1970) 680–685.
[16] J.H. Morrissey, Silver stain for proteins in polyacrilamine gels: a modified procedure with enhanced uniform sensitivity, Anal. Biochem. 117 (1981) 307–310.
[17] M.L. Price, L.G. Butler, Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain, J. Agric. Food Chem. 25 (1977) 1268–1273.
[18] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.
[19] W.J. Wolf, R.E. Peterson, M.L. Schaer, Preparative reversed-phase high-performance liquid chromatography of soybean proteins and characterization of fractions by gel electrophoresis, J. Agric. Food Chem. 40 (1992) 1809–1816.
[20] C.S. Farinas, A. Leite, E.A. Miranda, Aqueous extraction of recombinant human proinsulin from transgenic maize endosperm, Biotechnol. Prog. 21 (2005) 1466–1471.
[21] A.J. Charlton, N.J. Baxter, M.L. Khan, A.J.G. Moir, E. Haslam, A.P. Davies, M.P. Williamson, Polyphenol/peptide binding and precipitation, J. Agric. Food Chem. 50 (2002) 1593–1601.
[22] T.J. Menkhaus, Y. Bai, C. Zhang, Z.L. Nikolov, C.E. Glatz, Considerations for the recovery of recombinant proteins from plants, Biotechnol. Prog. 20 (2004) 1001–1014.
[23] N.C. Nielsen, Y.W. Nam, Soybean globulins, in: P.R. Shewry, R. Casey (Eds.), Seed Proteins, Kluwer Academic Publishers, Dortrecht, 1999, pp. 286–289.