

Allergenicity of main celery allergen rApi g1 and high-pressure treatment

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The structural changes and allergenicity of recombinant main celery allergen rApi g1 caused by the high pressure were studied. We have treated the buffer solutions of rApi g1 by high pressure at 500 MPa for pressure holding times 10 and 20 min and holding time temperatures 30 °C, 40 °C and 50 °C. The structural changes were studied by circular dichroismus (CD) spectra. The allergenic reaction of the rApi g1 was tested by Western blot analysis. The greatest changes of the structure were found at samples treated by 500 MPa at 50 °C. The samples treated at this temperature at pressure levels 400, 450 and 500 MPa and held for 10 and 20 min showed that protein structure changes are positively correlated with pressure. Western blot analysis evidenced that a pressure of 500 MPa held for 10–20 min at temperatures 30–50 °C did not change the allergenicity of the rApi g1 protein when compared with the untreated sample.

Keywords: celery; Api g1; allergen; high pressure

1. Introduction

An allergic reaction to celery can lead to anaphylactic shock. Therefore, celery has to be declared on food labels wherever its presence cannot be avoided. This liability motivates food producers to find methods for decreasing or inactivating the allergens in celery products. Classical heat preservation and also newly-emerging non-thermal processing methods such as high-pressure treatment (HPT) have had to be tested for their effect on the inactivation of allergic reactions to celery. Only heat processing was found to be a relatively successful method for decreasing the effects of celery allergens [1]. Cooking celery for 76 min at 100 °C did not completely remove the allergic reaction of sensitized patients [2]. A systematic study of the inactivation of the main celery allergen Api g1 by high pressure has not been done yet.

Recently, Scheibenzuber [3] and Meyer-Pittroff and Behrendt [4] presented a study suggesting that the allergenicity of apple slices can be inactivated by HPT. Meyer-Pittroff et al. [5] patented a method using HPT on apple slices to remove symptoms of oral allergy syndrome (OAS) in

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allergic patients. We were unable to confirm recently [6] that HPT alone is capable of removing or decreasing the allergenicity of Mal d1 or apple juice.

In most studies, HPT was used to assist or intensify the enzymatic hydrolysis reaction and production of peptides with lowered or removed allergenicity of proteins [7–13]. In [14,15], it was found that HPT itself has a minimal effect on the allergenicity of bovine gamma globulin or major rice allergens.

These results motivated us to study the separated effect of HPT without interaction with enzymes presented in the natural plant matrix. Therefore, we have studied the structural changes and allergenicity of pure Api g1 buffer solution as affected by high pressure levels, holding time and temperature.

2. Materials and methods

2.1. *rApi g1 solutions preparation*

Two milligrams of recombinant celery allergen (lyophilized rApi g1, Biomay, Austria) were diluted in 10 ml of phosphate buffer solution (0.01 M, $K_2HPO_4 \cdot 3H_2O$ in distilled water; pH adjusted to 7.4 with 0.1 N HCl). The diluted samples were stored in aliquots at -30°C . Before use, aliquots were carefully thawed at 5°C in a refrigerator. It should be considered that the buffer used was not pressure stable, and pH lowered during pressurization.

2.2. *CD electron spectroscopy*

Allergen rApi g1 structure changes were tested by circular dichroism electron spectroscopy (ECD). This work was done at the Institute of Chemical Technology in Prague, using a J-810 spectropolarimeter (Jasco, Japan). The sample was placed in a crystal glass flat cell, thickness of optical environment 1 mm, and carefully thermostated to 5°C . The spectral range was studied at wavelengths between 185 and 260 nm.

2.3. *Western bolt (WB) test description*

The serum of patients that exhibited a positive reaction to celery was used in the Western blot test. Anti-human IgE (produced in goats), anti-goat IgG–biotin (rabbits), conjugate streptavidin-peroxidase and other chemicals for electrophoresis came from Sigma–Aldrich. Samples were thawed carefully before testing. Further preparation of samples was completed according to Lämmli [16]. The SDS–PAGE electrophoresis protocol was used for protein separation. After electrophoresis gels were placed into transfer buffer (pH = 8.3); then, the gels were placed onto a membrane with the pooled plasma (three patients: DD four parts, SM three parts, BP three parts) and placed into a blotting chamber. Blotting conditions were: buffer glycine-methanol (pH = 8.3), constant current 350 mA and time of transfer = 3 h. A detailed description of the methodology is provided in our research report [17].

2.4. *High-pressure treatment*

An isostatic press (Zdas CYX 6/0103) with a chamber volume of 2 liters was used. Drinking water was used as the pressure-transmitting medium. An endpoint strategy was used to eliminate the influence of compression heating on samples. Because samples had water as their main component, we were able to use the rule 3°C per 100 MPa as the basis for our calculations. The vessel was

preheated to the final temperature predicted from the calculations. The samples and water were preheated to the starting temperature and placed into the chamber. Pressure-up time was 60 s for 500 MPa and pressure release time was about 3 s. The desired holding temperature was achieved using this method. This was verified by preliminary experiments, during which thermocouples were placed in the chamber in the same sample configuration used for the allergen treatment. Celery recombinant allergen rApi g1 solutions were treated at 500 MPa and held at 30 °C, 40 °C and 50 °C for 10 and 20 min. These samples, together with an untreated sample, were studied by

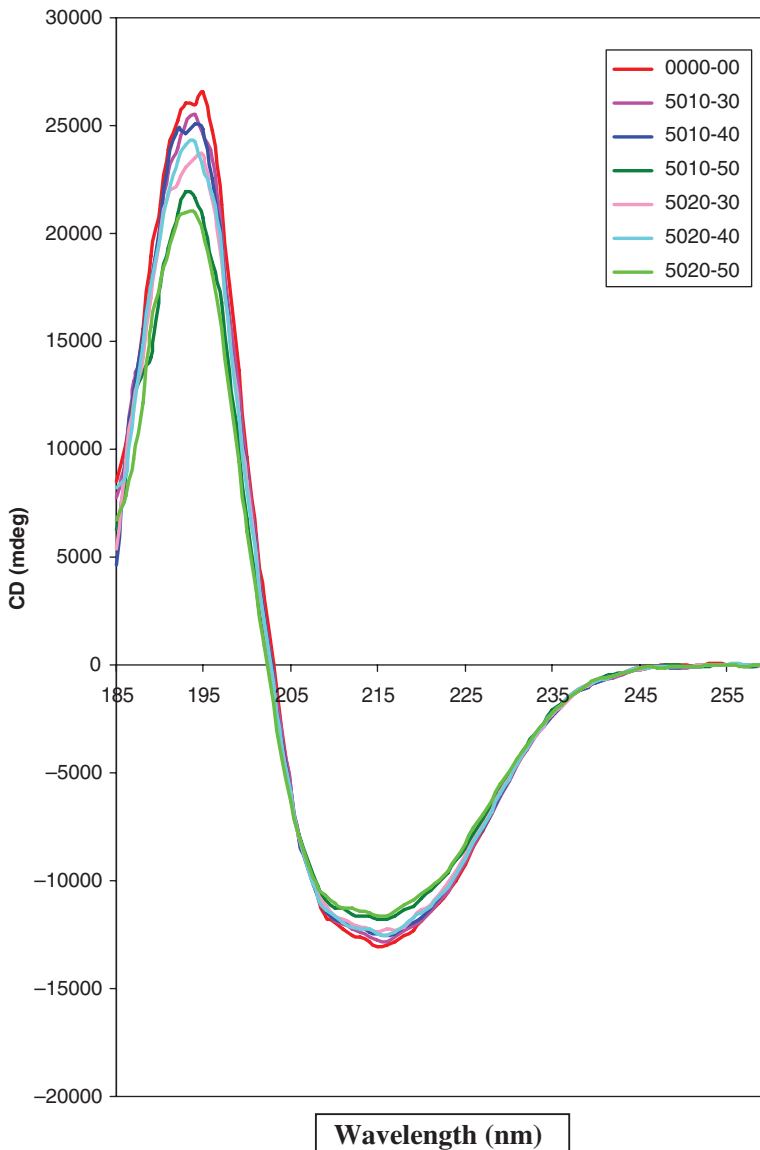


Figure 1. Electron CD spectra of HP-treated solutions of rApi g1: influence of temperature during pressure holding time; Legend: 0000-00, untreated sample; 5010-30, 500 MPa held for 10 min at 30 °C; 5010-40, 500 MPa held for 10 min at 40 °C; 5010-50, 500 MPa held for 10 min at 50 °C; 5020-30, 500 MPa held for 20 min at 30 °C; 500-20-40, 500 MPa held for 20 min at 40 °C; 5020-50, 500 MPa held for 20 min at 50 °C.

ECD spectra and the Western blot method. Other rApi g1 solution samples were treated at 400, 450 and 500 MPa and held for 10 and 20 min at 50 °C. The structure changes of these samples were studied by ECD spectra.

2.5. Statistics

The ECD spectra were measured by 10 successive scans on given sample and mean spectrum was calculated by the instrument.

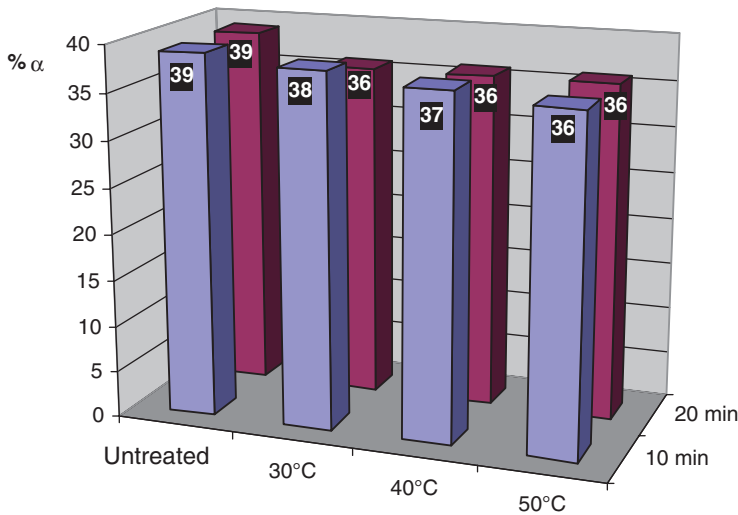


Figure 2. Percentage of α -structure in pressure (500 MPa)-treated rApi g1 buffer solutions: influence of temperature and holding time.

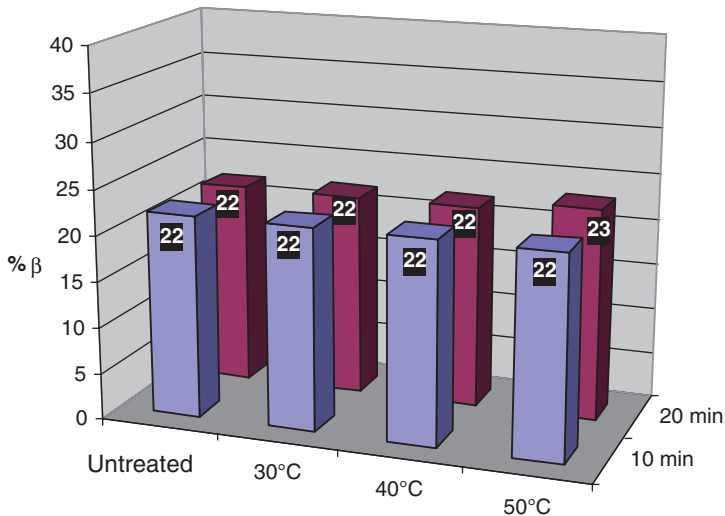


Figure 3. Percentage of β -structure in HP (500 MPa)-treated samples of rApi g1 buffer solutions: influence of temperature.

3. Results and discussion

3.1. Structural changes of allergen solutions

3.1.1. Influence of temperature at HPT

3.1.1.1. *ECD spectra changes.* The ECD spectra of rApi g1 10 mM phosphate buffer solutions are given in Figure 1. The influence of temperature held during pressure treatment is apparent.

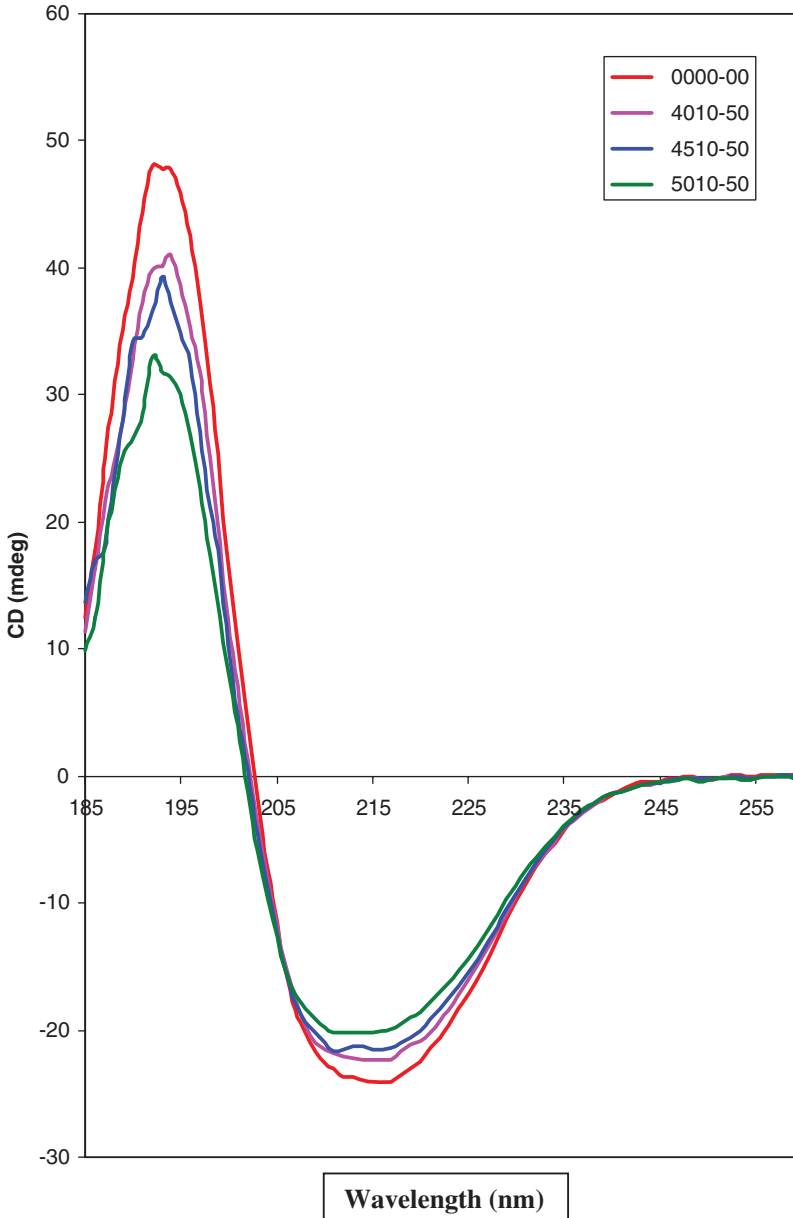


Figure 4. ECD spectra of pressure-treated rApi g1 buffer solutions: influence of pressure at holding time 10 min and temperature 50 °C. Legend: 0000-00, untreated sample; 4010-50, 400 MPa held for 10 min at 50 °C; 4510-50, 450 MPa held for 10 min at 50 °C; 5010-50, 500 MPa held for 10 min at 50 °C.

The positive maximum at 193 nm and negative maximum at 215 nm decreased with increasing temperature. The influence of the holding time 10 and 20 min is not apparent. The greatest change of spectra was found for the temperature 50 °C.

3.1.1.2. *Assessment of secondary structure changes.* Structural changes were assessed by the software Dicroprot and method K2D (neuron network); results are given in Figures 2 and 3. Slight decrease in percentage of α -helical structure with increased temperature at HPT is apparent. This decrease is more visible at 20 min holding time. Percentage of β -sheet structure is not changed with changing temperature at HPT and holding time.

3.1.2. Influence of pressure at temperature 50 °C

3.1.2.1. *ECD spectra changes.* The ECD spectra of rApi g1 10 mM phosphate buffer solutions are given in Figure 4. Increasing pressure at given holding time 10 min decreases the positive maximum at 193 nm and negative maximum at 215 nm. The greatest change of spectra when compared with the untreated sample was found at 500 MPa pressure (see also Table 1).

Table 1. Intensity of CD spectra at given wave lengths as a function of pressure (CD values read from smoothed spectra mean curve).

Pressure (MPa)	Intensity of CD band at 193 nm θ (mdeg)	Relative intensity of CD band at 193 nm (%)	Intensity of CD band at 215 nm θ (mdeg)	Relative intensity of CD band at 215 nm (%)
0	46.29	100	-23.96	100
400	38.93	84.1	-22.31	93.1
450	36.40	78.6	-21.48	89.6
500	30.68	66.3	-20.25	84.5

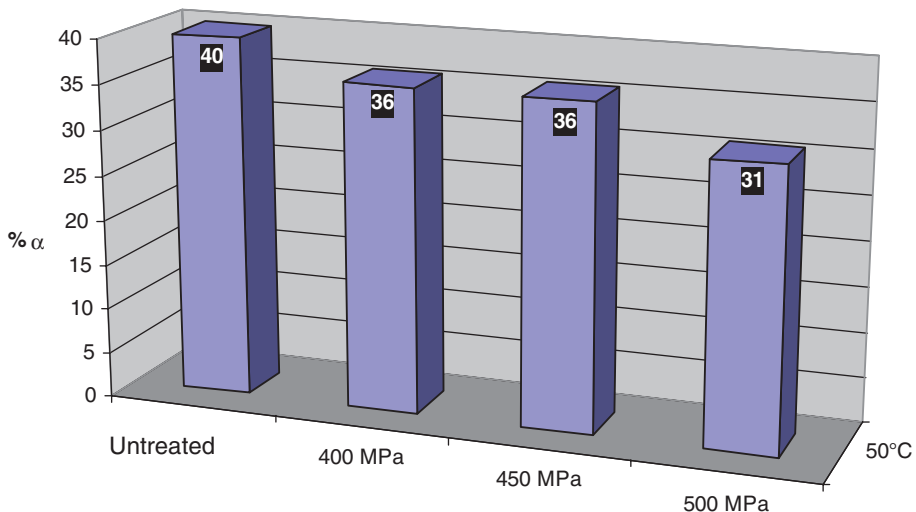


Figure 5. Percentage of α -structure at pressure-treated rApi g1 buffer solutions: influence of pressure level (holding time 10 min).

3.1.2.2. *Assessment of secondary structure changes.* α -helical structure percentage slightly decreases with increasing pressure (Figure 5). Substantial β -sheet structure percentage change can be observed only for sample pressurized at 500 MPa (Figure 6).

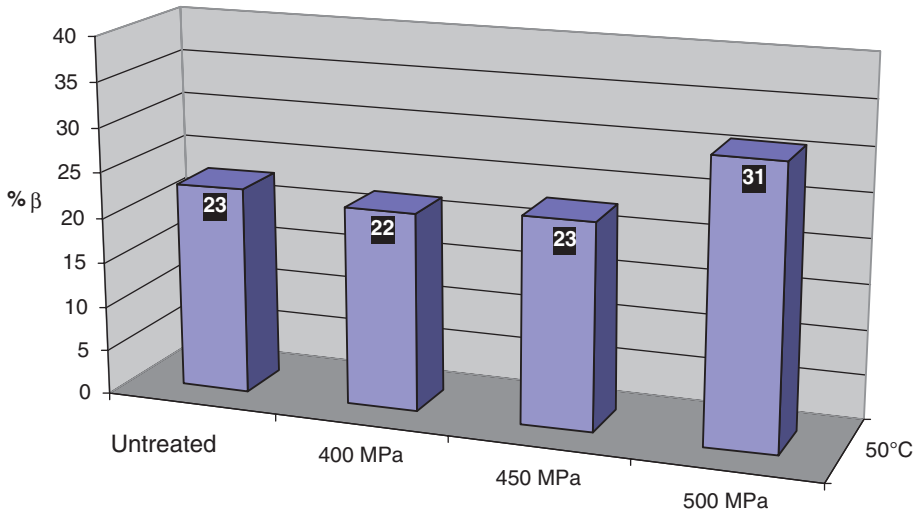


Figure 6. Percentage of β -structure in pressure-treated samples of rApi g1 buffer solutions: influence of pressure level (holding time 10 min).

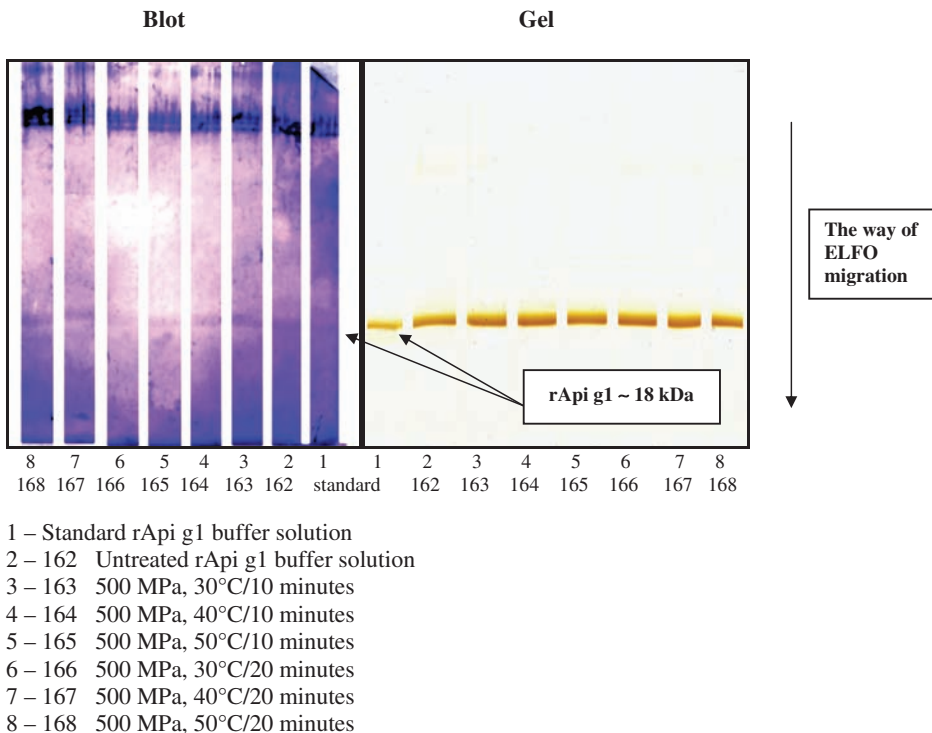


Figure 7. Results of the Western blot test of samples of pressurized rApi g1 buffer solutions.

3.2. Results of WB of allergen solutions

Western blot test results of pressure treated rApi g1 10 mM phosphate buffer solutions are displayed in Figure 7. Electrophoretic gels evidenced presence of protein rApi g1 in all samples. Blots visualized weakly apparent bands at lines of all pressure treated samples (162–168) and also untreated and standard samples. This is the evidence that used HPT at applied parameters (500 MPa, holding time 10–20 min, temperatures 30–50 °C) was not able to change the allergenicity of rApi g1 as evaluated by the WB analysis.

4. Conclusions

HPT caused weak changes of the structure of rApi g1 allergen as observed in ECD spectra. The greatest structure changes were observed at the highest applied temperature of 50 °C held during HPT of 500 MPa. The ECD spectra changes generated at these HPT conditions corresponded to the lowered percentage of the α -helical structure. The influence of pressure holding time was minimal.

Different pressures applied at 50 °C caused substantial changes of the structure as observed by ECD spectra. Increasing pressure causes lowering percentage of α -helical structure and the increasing β -sheet structure. It can be concluded that HPT caused substantial conformational changes in rApi g1 protein structure.

In spite of these structural changes, the applied HPT conditions (500 MPa, 10–20 min, at 30–50 °C) did not lead to the lowering the allergenicity as evidenced in the Western blot test.

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