

Allergenicity of main celery allergen rApi g1 and high pressure treatment

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Introduction

The allergic reaction to celery can lead up to the 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 inactivate the allergens in celery products. Classical heat preservation and also emerging non-thermal processing methods such as high pressure treatment (HPT) had to be tested for its effect in inactivation of allergic reactions of celery. The only heat processing was found as relatively successful method for decreasing the celery allergens effects. But the systematic study of inactivation of main celery allergen Api g1 by high pressure was not done yet. This situation motivated us to study the effect of HPT on structural changes and allergenicity of pure Api g1 buffer solution. The influence of the high pressure level, holding time and temperature was investigated.

Material and methods

Material:

rApi g1 buffer solution preparation

Two mg of recombinant celery allergen (lyophilised rApi g1, Biomay, Austria) were diluted in 10 ml of phosphate buffer solution (0.01 M, K₂HPO₄·3H₂O in distilled water; pH adjusted to 7.4 with 0.1N HCl). The diluted samples were stored in aliquots at -30°C. Before use, aliquots were carefully thawed at 5°C in a refrigerator.

Methods:

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 crystal glass flat cell having thickness of optical environment 1 mm, and carefully thermostated to 5°C. The spectral range was studied at wavelengths between 185 – 260 nm.

Western blot 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 done according to Lämml. 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 4 parts, SM 3 parts, BP 3 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 hours.

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 end point 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 calculations. The samples and water were preheated to the starting temperature and placed into the chamber. Pressure-up time was 60 seconds for 500 MPa, pressure release time was about 3 seconds. The desired holding temperature was achieved using this method. This was verified by preliminary experiments during which thermometers were placed in the chamber in the same sample configuration used with the allergen treatment. Celery recombinant allergen rApi g1 solutions were treated at 500 MPa and held at 30, 40 and 50°C for 10 and 20 minutes. These samples together with untreated sample were studied by CD spectra and Western blot method. Other rApi g1 buffer solution samples were treated at 400, 450 and 500 MPa and held for 10 and 20 minutes at 50°C. The structure changes of these samples were studied by CD spectra.

Results

Structural changes of allergen solutions

Influence of temperature at HPT

ECD spectra changes

Electron circular dichroism (ECD) spectra of rApi g1 buffer solutions are given in Fig. 1. There is apparent the influence of temperature held during pressure treatment. 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 minutes is not apparent. The greatest change of spectra was found for the temperature 50°C.

Assessment of secondary structure changes

Structural changes were assessed by the software Dicroprot and method K2D (neuron network). Results are given on Fig. 2 and 3. Slight decrease of percentage of α -helical structure with increased temperature at HPT is apparent. This decrease is more visible at 20 minutes holding time. Percentage of β -sheet structure is not changed with changing temperature at HPT and holding time.

Influence of pressure at temperature 50°C

ECD spectra changes

ECD spectra of rApi g1 buffer solutions are given in Fig. 4. Increasing pressure at given holding time 10 minutes decreases positive maximum at 193 nm and negative maximum at 215 nm. Greatest change of spectra compared to untreated sample was found for pressure 500 MPa, 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

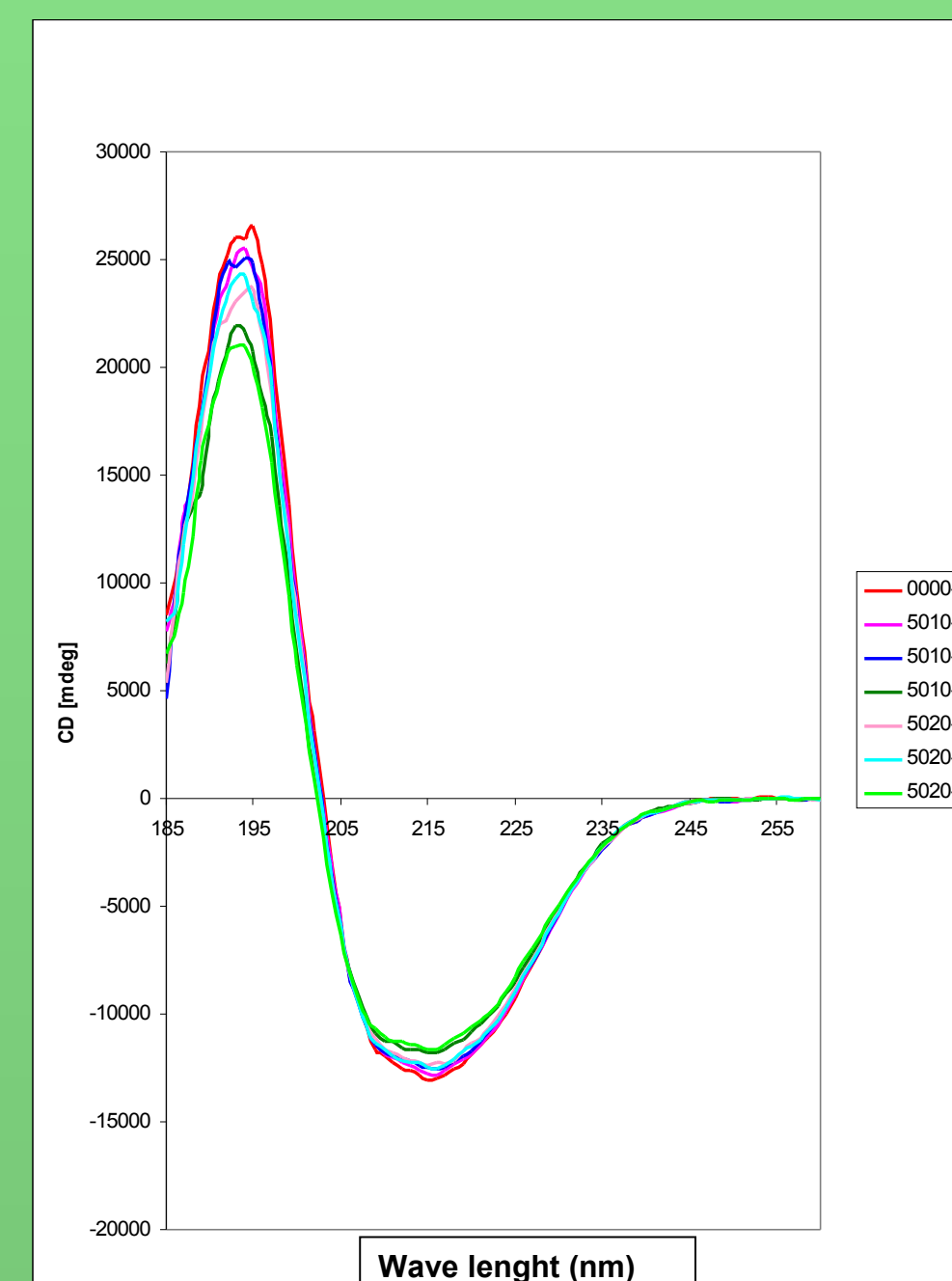


Fig. 1 Electron CD spectra of HP treated solutions of rApi g1- influence of temperature during pressure holding time

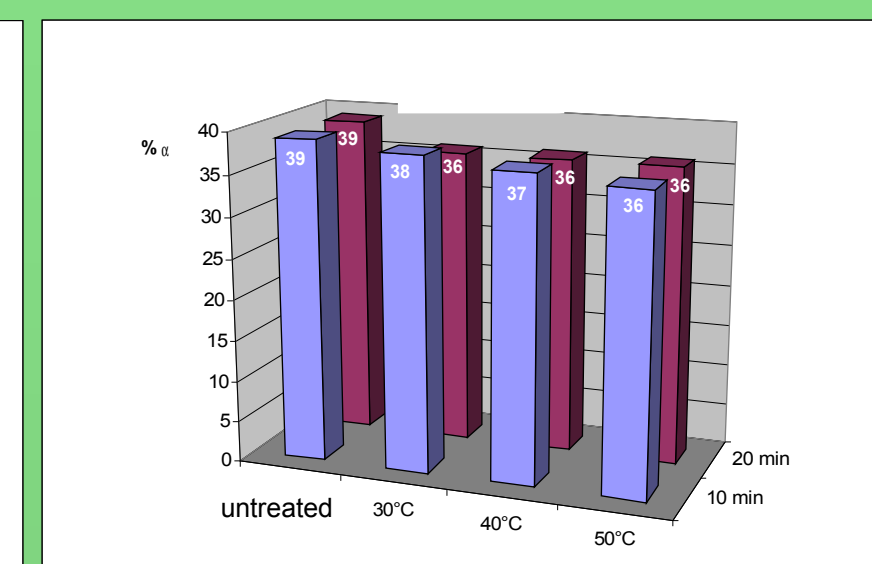


Fig. 2 Percentage of α -structure in pressure treated rApi g1 buffer solutions – influence of temperature and holding time

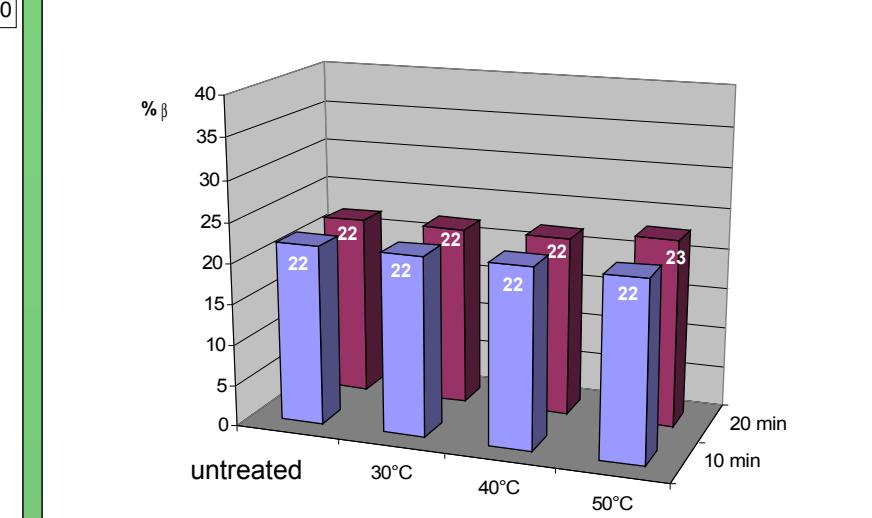


Fig. 3 Percentage of β -structure in HP treated samples of rApi g1 buffer solutions – influence of temperature

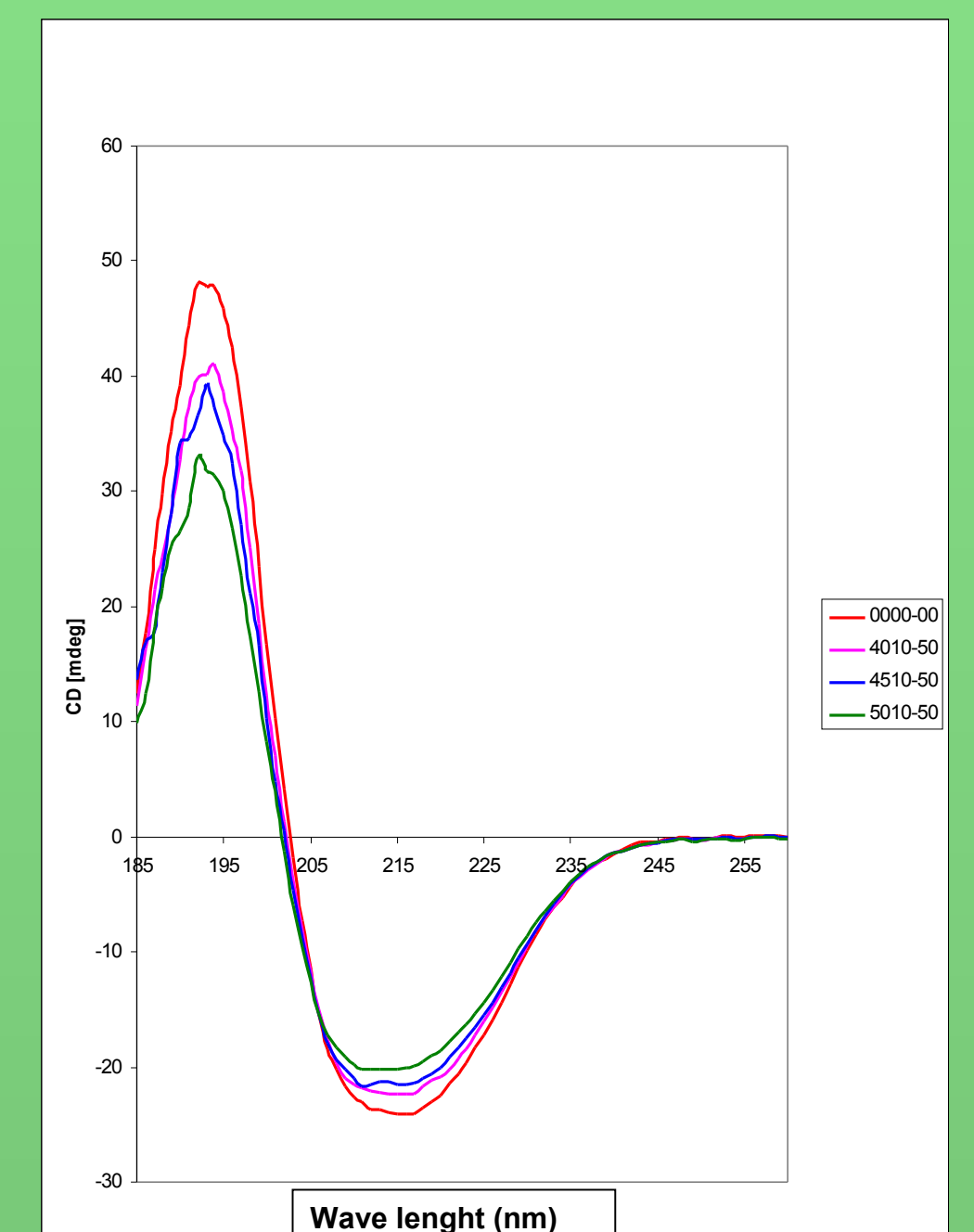


Fig. 4 ECD spectra of pressure treated rApi g1 buffer solutions – influence of pressure at holding time 10 minutes and temperature 50°C

Assessment of secondary structure changes

α -helical structure percentage slightly decreases with increasing pressure, see Fig. 5. Substantial β -sheet structure percentage increase can be observed only for sample pressurized at 500 MPa, see Fig. 6.

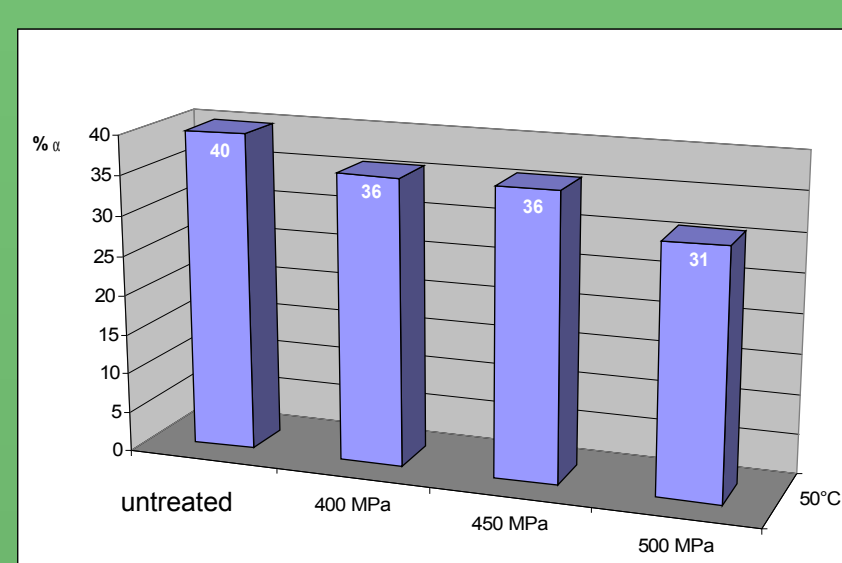


Fig. 5 Percentage of α -structure at pressure treated rApi g1 buffer solutions – influence of pressure level

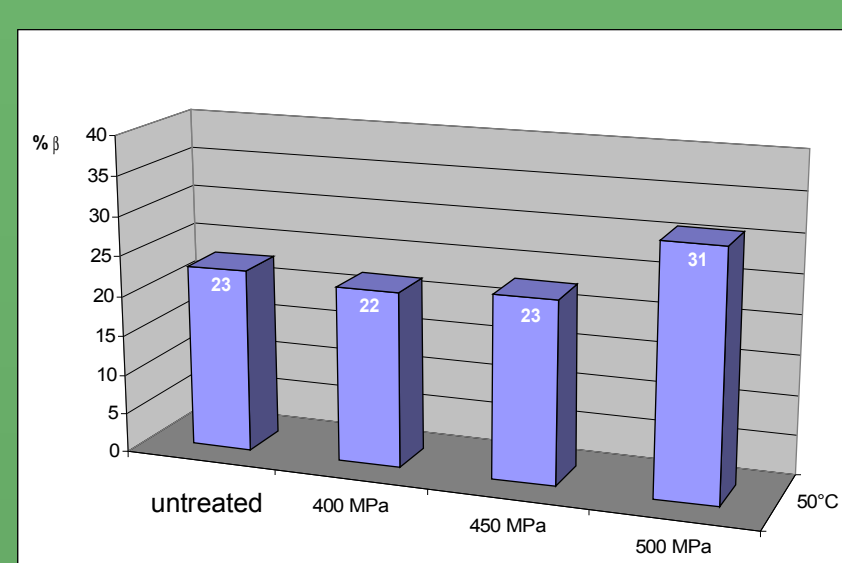
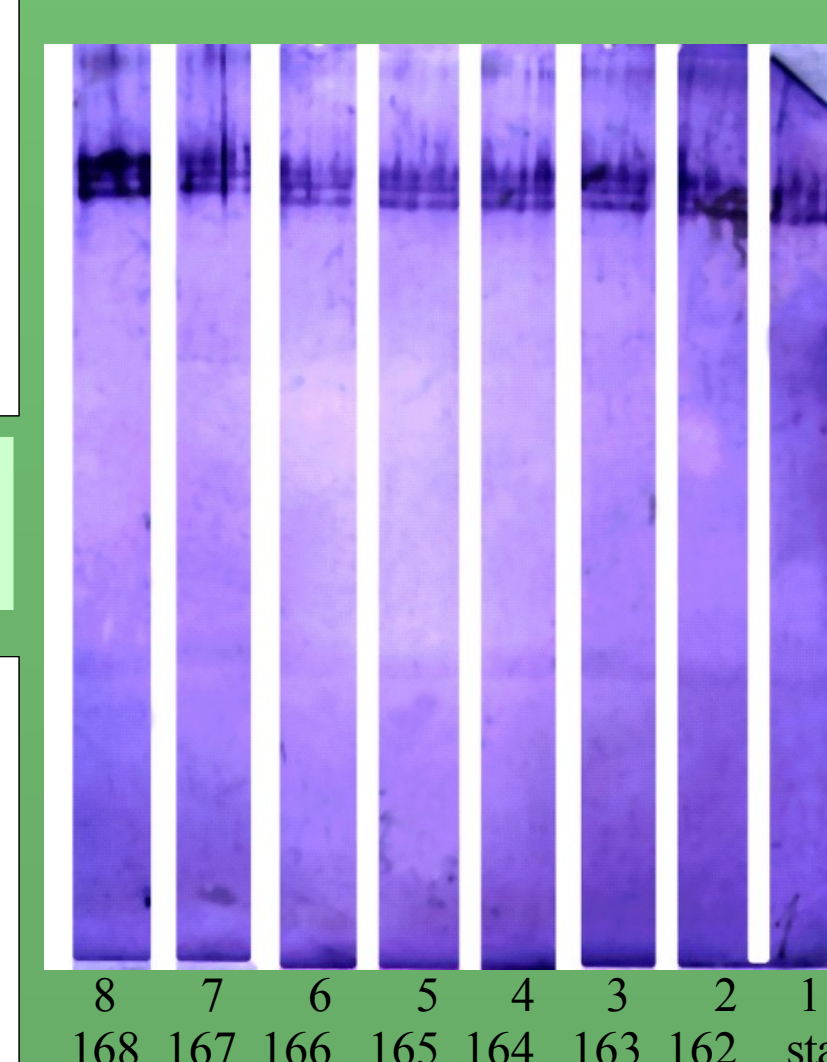


Fig. 6 Percentage of β -structure in pressure treated samples of rApi g1 buffer solutions – influence of pressure level

Blot



- 1 – standard rApi g1 buffer solution
- 2 – 162 untreated rApi g1 buffer solution
- 3 – 163 500 MPa, 30°C/10 minutes
- 4 – 164 500 MPa, 40°C/10 minutes
- 5 – 165 500 MPa, 50°C/10 minutes
- 6 – 166 500 MPa, 30°C/20 minutes
- 7 – 167 500 MPa, 40°C/20 minutes
- 8 – 168 500 MPa, 50°C/20 minutes

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

Results of WB of allergen solutions

Western blot test results of pressure treated rApi g1 buffer solutions are displayed in Fig. 7. Electrophoretic gels evidenced the 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 sample. This is the evidence that used high pressure treatment at applied parameters (500 MPa, holding time 10 – 20 minutes, temperatures 30-50°C) was not able to change the allergenicity of rApi g1.

Conclusions

High pressure treatment caused weak changes of the structure of rApi g1 allergen as observed in ECD spectra. The greatest structure changes were observed at highest applied temperature 50°C held during high pressure treatment of 500 MPa. The CD 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 CD 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 minutes, at 30 – 50°C) did not lead to the lowering the allergenicity as evidenced in Western blot test.

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