

## Allergenicity of main birch allergen rBet v1 and high-pressure treatment

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The aim of our study was to determine the influence of high-pressure treatment on the structure and allergenicity of recombinant rBet v1, the main birch allergen and birch pollen extract. We treated the buffered solutions of rBet v1 and birch pollen extract with high pressure (450–550 MPa) for a period of 10 min at temperatures between 30 and 50 °C. The structural changes in rBet v1 were studied using circular dichroism spectra. The greatest changes in the rBet v1 structure were found in the samples treated at 450 MPa at 30 °C. The samples treated at 500 and 550 MPa at 30 °C (10 min) did not show any structural changes. The allergenic reaction to rBet v1 and the birch pollen extract was tested using Western blot analysis. Pressures of 450–550 MPa (10 min) at temperatures of 30, 40 and 50 °C did not change the allergenicity of the rBet v1 protein or the birch pollen extract when compared with untreated samples.

**Keywords:** birch pollen extract; rBet v1; allergen; high pressure

### 1. Introduction

Central Europe is seeing an increasing number of inhabitants allergic to birch pollen [1]. They suffer from allergic rhinitis, conjunctivitis, eczema and allergic asthma. More than 50% also have oral allergy syndrome (OAS). These effects have been described after the consumption of fresh fruits or vegetables, including apples [2], and are most common in Central and Northern Europe.

Meyer-Pittroff et al. [3] and Scheibenzuber [4] presented studies suggesting that the allergenicity of apple slices can be inactivated by high-pressure treatment (HPT). Meyer-Pittroff et al. [3] patented a HPT method for apple slices to remove symptoms of OAS in allergic patients. These results motivated us to study the influence of HPT not only on the apple allergen, Mal d1 [5], but also on the main birch allergen, Bet v1. The main goal of this work was to find the optimal HPT parameters (pressure, holding time and temperature) for the most effective inactivation of Bet v1 and birch pollen extract.

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## 2. Materials and methods

### 2.1. *rBet v1 solutions preparation*

Two milligrams of recombinant birch allergen (rBet v1; Biomay, Austria) were diluted in 10 ml of buffer solution (10 mM  $K_2HPO_4 \cdot 3H_2O$  in distilled water; pH adjusted to 7.0 with 0.1 N HCl). The diluted samples were stored in aliquots at  $-30^\circ C$ . Before use, the aliquots were carefully thawed at  $5^\circ C$  in a refrigerator.

### 2.2. *Allergen extraction from nature birch pollen*

We obtained 200 g of dried birch pollen (Lesny-Forest Ltd., Chlumec 26, 373 41 Hluboká nad Vltavou) with a purity of 60%. Sevapharma Ltd., Prague, performed the extraction and standardized the concentration to 34,000 protein nitrogen unit (PNU) and an activity of 174,579 unit of standard quality (USQ) [1 PNU = 0.00001 mg PN in 1 ml of allergen extract; USQ: biological activity of 1000 USQ is the activity which causes a wheal (diameter 5.5 mm), from prick tests on 20 allergic patients].

### 2.3. *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 the samples. For samples having water as their main component, we used  $3^\circ C/100$  MPa as the basis for our calculations. The vessel was preheated to the desired temperature. The samples and the water necessary to pre-fill the chamber were preheated to the starting temperature predicted from calculations and placed into the chamber. The pressure-up time was 60 s for 500 MPa; the pressure release time was about 3 s. The desired holding temperature was achieved using this method. This was verified by preliminary experiments during which the thermometers were placed in the chamber in the same sample configuration used for the allergen. The birch pollen extract and the rBet v1 solutions were treated at 400–550 MPa and held at 30, 40 and  $50^\circ C$  for 10 min.

### 2.4. *WB test description*

The serum of the patients that exhibited a positive reaction to birch and Bet v1 allergen was used in the Western blot (WB) test. Anti-human IgE (produced in goats), anti-goat IgG-biotin (rabbits), conjugate streptavidin-peroxidase and other chemicals for electrophoresis came from Sigma–Aldrich. The samples were thawed carefully before testing. Further preparation of samples was done according to Lämmli [6]. The SDS–PAGE electrophoresis protocol was used for protein separation. After electrophoresis, the gels were placed into the transfer buffer (pH 8.3); then the gels were placed onto a membrane with the patients' plasma and placed in a blotting chamber. The blotting conditions were as follows: buffer glycine–methanol (pH 8.3), constant current 350 mA, and the time of transfer was 3 h.

### 2.5. *CD electron spectroscopy*

Allergen rBet v1 structural changes were tested using circular dichroism (CD) electron spectroscopy. 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 flat crystal glass 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. The data evaluations were completed following the procedure described in [7] and [8].

### 3. Results and discussion

#### 3.1. Structural changes of allergen solutions

The CD spectra of rBet v1 solutions treated at different pressures and temperatures (10 min) are shown in Figures 1–3. The spectra changes were generally minimal. The greatest change was observed for the sample treated at 450 MPa at 30 °C (see Figure 1). Other pressure levels tested did not show any substantial changes in the CD spectra (see Figures 2 and 3).

#### 3.2. Results of WB of allergen solutions and birch pollen extract

Allergic reactivity of HPT rBet v1 solutions were studied as a function of holding temperatures (30, 40 and 50 °C) and pressures (500 and 550 MPa); the holding time for all the samples was 10 min (see Figure 4). All the samples tested, as well as the control samples, exhibited reactivity with the serum of sensitized patients without regard to temperature or pressure used. The HPT of rBet v1 solutions failed to change the allergic reactions.

The allergic reactivity of HPT birch pollen extract was also studied as a function of holding temperatures (30, 40 and 50 °C) and pressures (450, 500 and 550 MPa); the holding time was 10 min (see Figure 5). The HPT applied under these conditions failed to change the allergenic reactions in the Western blot test.

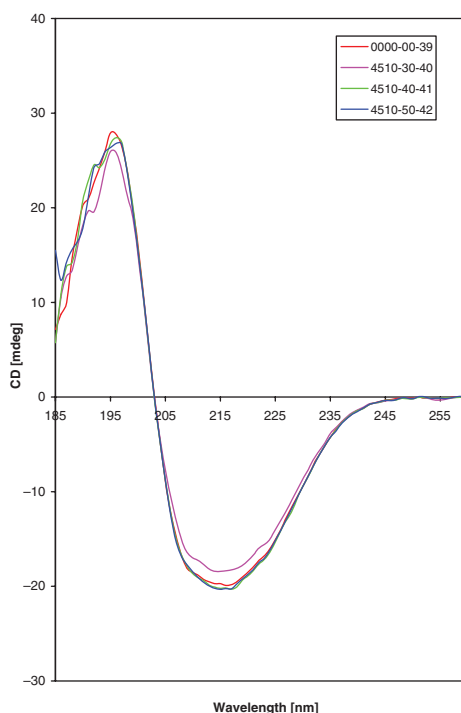


Figure 1. Electron circular dichroism (ECD) spectra of allergen rBet v1 treated at 450 MPa for 10 min at temperatures of 30, 40 and 50 °C compared with the untreated sample.

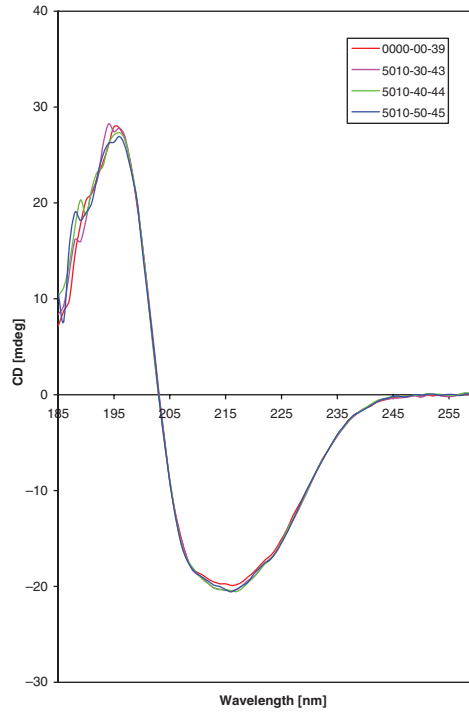


Figure 2. ECD spectra of allergen rBet v1 treated at 500 MPa for 10 min at temperatures of 30, 40 and 50 °C compared with the untreated sample.

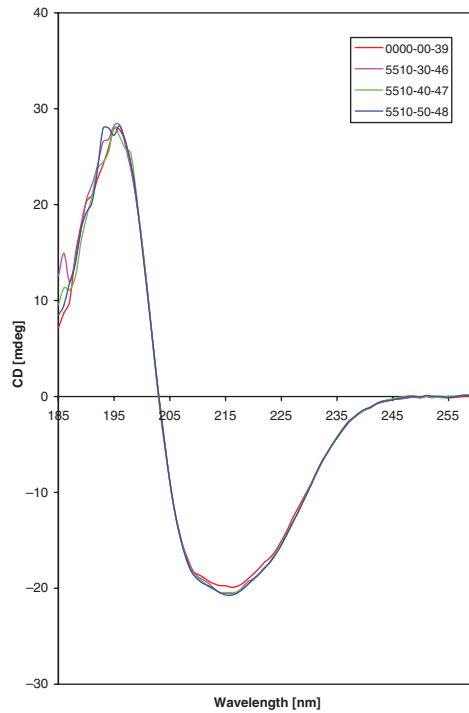
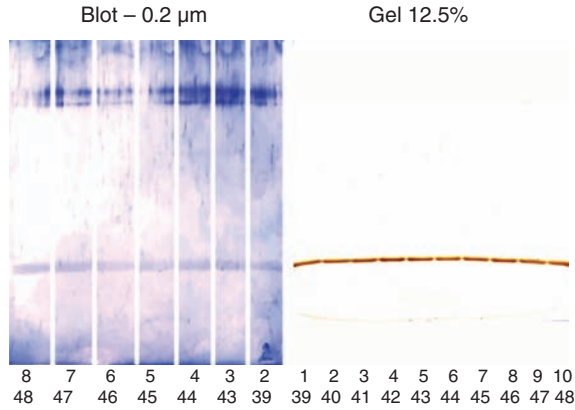


Figure 3. ECD spectra of allergen rBet v1 treated at 550 MPa for 10 min at temperatures of 30, 40 and 50 °C compared with the untreated sample.



Pressure 500 MPa samples 43, 44, 45 holding temperature 30, 40 a 50°C;  
 Pressure 550 MPa samples 46, 47, 48 holding temperature 30, 40 a 50°C.

Figure 4. Influence of pressure and holding temperature on allergenicity of rBet v1 in the Western blot test.

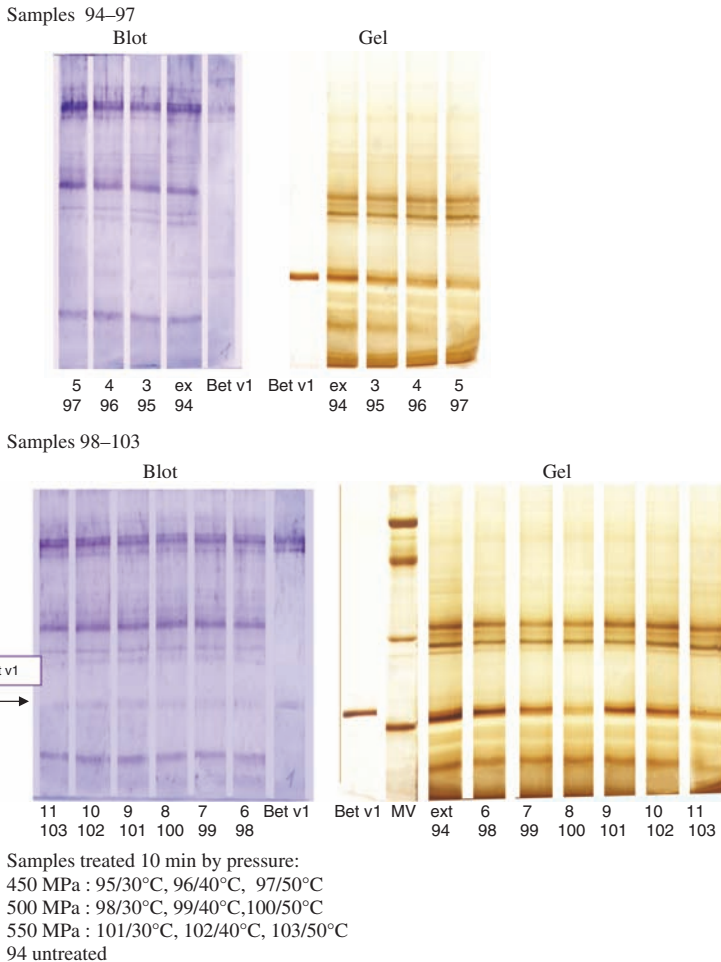


Figure 5. Influence of pressure and holding temperature on allergenicity of birch pollen extract tested by the Western blot.

## 4. Conclusions

In our study, the HPT applied to the ranges of pressure, temperature and holding times was not capable of altering the allergenicity of rBet v1 or birch pollen extract in solutions.

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