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Supplement of

Aggregation of ice-nucleating macromolecules from *Betula pendula* pollen determines ice nucleation efficiency

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S1 Background measurements

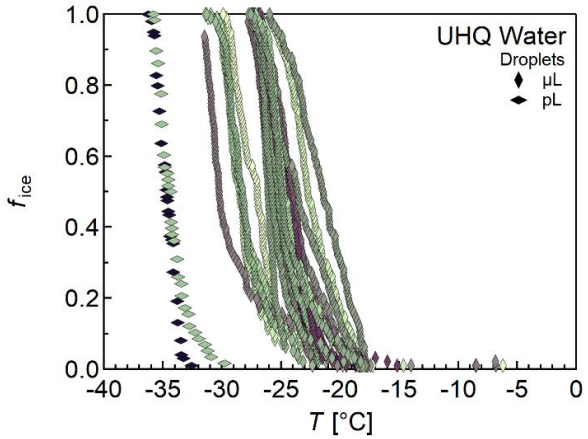

Figure S 1. Ice nucleation activity of background measurement for the µL- and the pL-droplet assay. The background for the µL-droplet assay starts as early as -15°C (with some single droplets starting even higher), but most blanks froze between -20°C and -30°C. The pL-droplet background starts at about -33°C, with a few droplets freezing at higher temperatures.

S2 Additional Information for untreated BPWW

- Pollen A: Harvested in March 2020 in České Budějovice, Czech Republic, (Pharmallerga, Czech Republic)
- Pollen B: Harvested in March 2019 in Galanta/Trnava/Senec, Slovakia, (Pharmallerga, Czech Republic).

Sample	Date of measurement	Prior to measurement
A0	12th Oct. 2021	stored at -20°C over night
A1	20th Jul. 2023	stored at -20°C over night
A2	19th Jul. 2023	measured immediately after preparation
B0	10th Dec. 2021	stored at -20°C over night
B1	20th Jul. 2023	measured immediately after preparation
B2	19th Jul. 2023	stored at -20°C over night

Table S1. Information of date of measurement of BPWW sample and handling prior to the measurement.

S3 Additional graphs

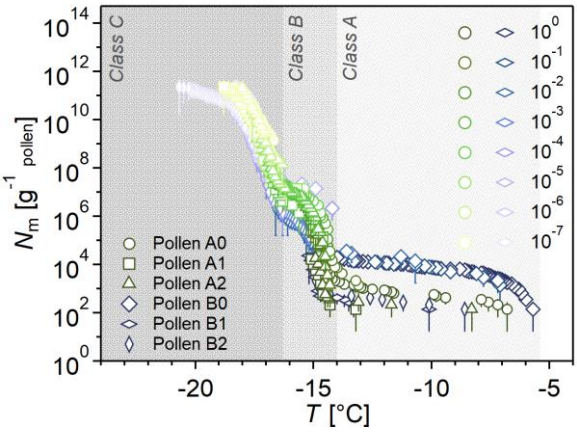

Figure S 2. Freezing spectra of INMs from *Betula pendula* pollen. Cumulative number $N_m(T)$ of INMs per gram *Betula pendula* pollen. Pollen from different growing regions: pollen A from the Czech Republic (Pharmallerga), pollen B from Slovakia (Pharmallerga). Ice nucleation activity above -15°C varies independently of the growing region. The legend on the left indicates the dilution (e.g., 10^{-1} equals a 1:10 dilution, one part sample to nine parts ultra-high quality (UHQ) water).

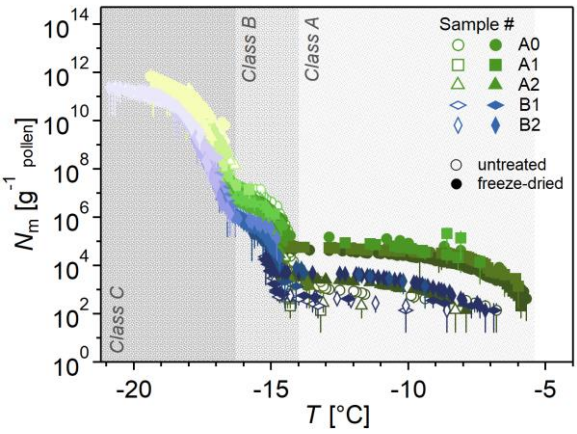

Figure S 3. Effect of freeze drying (experiment replications). Cumulative number $N_m(T)$ of INMs per gram *Betula pendula* pollen. The effect on ice nucleation activity varies from experiment to experiment, but ice nucleation activity always increases, and mostly, ice nucleation activity > -15°C is affected. Solid symbols mark untreated samples (non-freeze-dried), and hollow symbols mark freeze-dried samples. Green colors represent pollen A (Czech Republic), and Blue represents pollen B (Slovakia). Measurement A0 is the data shown in figure 2 of the manuscript.

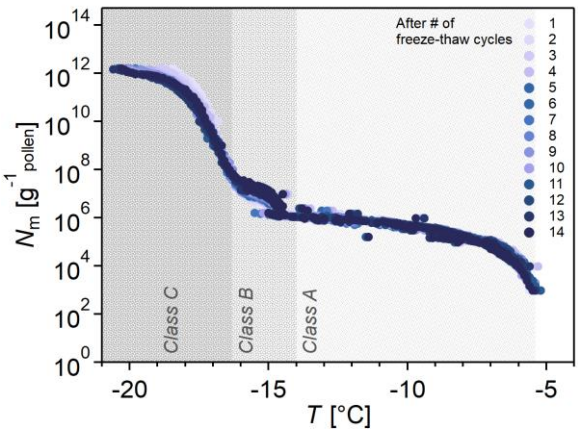

Figure S 4. Effect of freeze-thaw cycles (whole freezing spectrum). Cumulative number $N_m(T)$ of INMs per gram *Betula pendula* pollen (Pollen A0). In addition to figure 3, which showed only a zoomed in version of the full freezing spectrum.

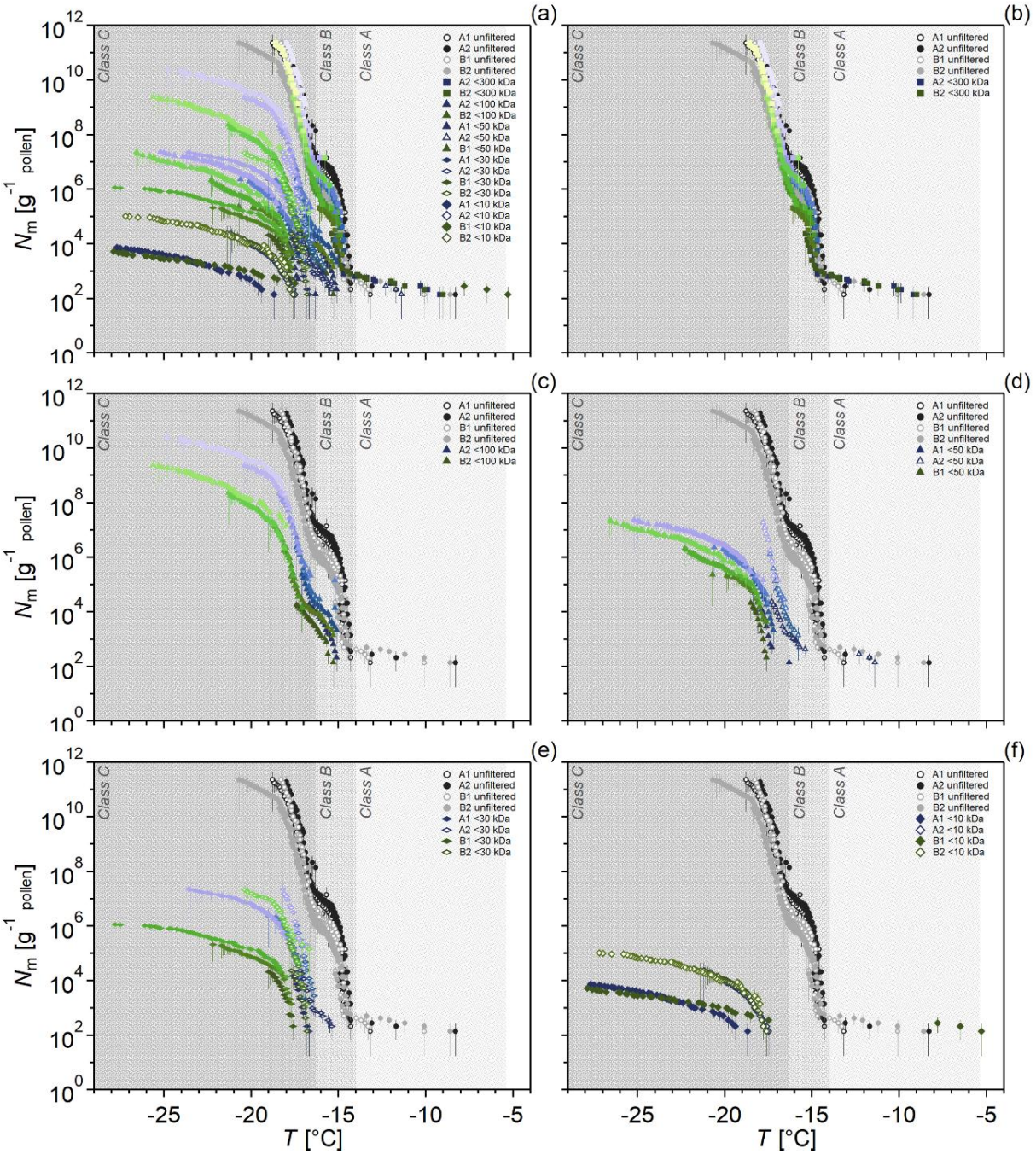

Figure S 5. Size-selective filtrations (experiment replications). Cumulative number $N_m(T)$ of INMs per gram *Betula pendula* pollen. (a) Showing all measurements, this includes filtrations series from two different *Betula pendula* pollen (A from the Czech Republic in blue tones, B from Slovakia in green tones) and two different BPWW batches each (1 and 2). The unfiltered samples are shown in black (A) and grey (B) in all plots. (b) – (d) Comparing unfiltered (220 nm filtered during BPWW preparation) samples to the 300 kDa (b), 100 kDa (c), 50 kDa (d), 30 kDa (e), and 10 kDa (f) filtrates. The 300 kDa and 100 kDa filtrations were only replicated once (A2 and B2) since the effect on the sample is only minor. When measuring sample B2 50 kDa filtrate, an error occurred.

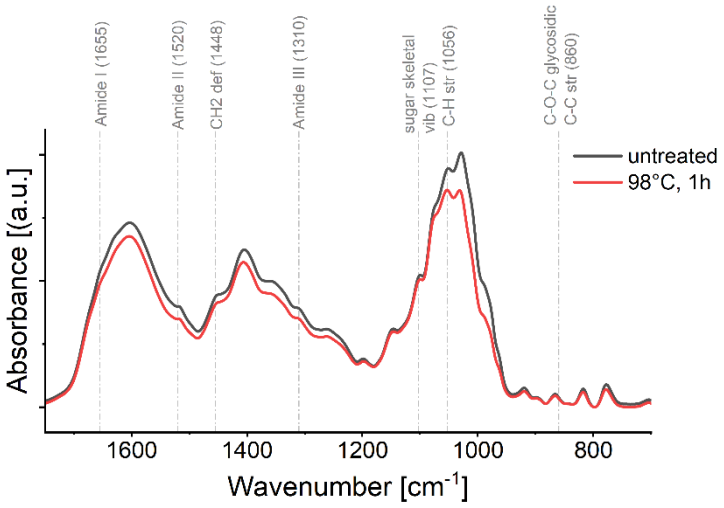

Figure S 6. Infrared spectrum of untreated and heat treated BPWW (Pollen A0). A selection of typical protein and polysaccharide bands has been marked (Pummer et al., 2013a).

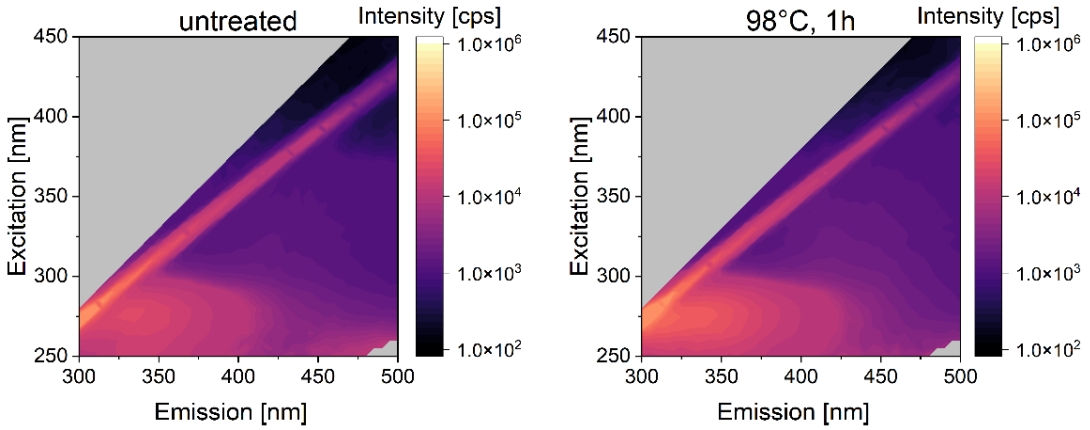

Figure S 7. Fluorescence excitation-emission measurement of untreated and heat treated BPWW (Pollen A0). The main signals are at excitation ~ 275 nm and emission at ~ 330 nm, indicating aromatic amino acids' presence. The diagonal signal is the Raman scattering caused by the solvent (water).