Comments to “Aggregation of ice-nucleating macromolecules from Betula pendula pollen determines ice nucleation efficiency” by Florian Wieland et al. (Manuscript ID: egusphere-2024-752).

Based on the twin-plate ice nucleation assay (TINA) and Vienna optical droplet crystallization analyzer (VODCA), the authors explored the ice nucleation activity of ice nucleating macromolecules (INMs) in Betula pendula pollens. The authors found three different types of INMs that caused freezing at -8.7°C, -15.7°C, and -17.4°C, respectively. The authors also found freeze drying, freeze-thaw cycles, and heat treat can alter the ice nucleation activity, which was attributed to size-varying aggregates. Overall, the science is interesting, and manuscript is written well. If the following comments can be fully addressed, the manuscript may be publishable in *Atmos Chem Phys.*

Major comments:

1. The droplets in TINA experiment are shaped by the well. However, the droplets in VODCA are shaped by surrounding aqueous phase. How the different morphology and contact angles of generated droplets will influence the measured ice nucleating activity?
2. The authors treated the BPWW at 40 °C, 78 °C and 98 °C for 1 h or 24 h respectively. What is the relevance of such high temperatures to ambient air especially the troposphere air?
3. Why the error bars of hydrodynamic radius R_h of untreated BPWW are significantly higher than other samples (Figure 6)?
4. Figure 1: in the left panel, the data points for pL droplets scattered significantly. One temperature corresponds to several N_m values, how to explain this?
5. Line 127: is the comment ‘VODCA is optimal for high INM concentrations at low temperatures, while TINA is better for low INM concentrations at higher temperatures’ reasonable?
6. Figure 4: it looks like the drying time duration significantly influence the N_m. Whether the chemical composition has been changed? FTIR data is preferred to confirm this point.
7. Figure 5: what is the relative fraction change of different size INMs for untreated and treated BPWW samples?