

# Protein extraction from cypress pollen with Minilys ESPCI, LSABM, Allergy & Environment, France

## CONTEXT

The common cypress pollen (*Cupressus sempervirens*) is becoming an increasing cause of respiratory allergy in Europe and some regions worldwide.

Because of its particular structural features and physico-chemical composition, cypress pollen is one of the most pollen difficult to analyze in terms of protein content and therefore allergens.

In aqueous media, the external wall (exine) cracks in a few minutes under the effect of swelling of the intine (inner wall) particularly rich in polysaccharide. Few proteins are then extracted in aqueous conditions.

The dry milling may be a good alternative for the extraction of cypress pollen proteins and to generate fragments of smaller sizes for experiments and ultra-structural analysis of immuno-reactivity [1].

## MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-003 (1.4 mm ceramic beads).
- Sample: 100 mg of *Cupressus sempervirens* pollens.

## PROTOCOL

- Minilys: 5000 rpm, 1x30s and 3x30sec (15s break).
- Analysis: Microscopic observation (X40) / 1-D (SDS-PAGE) immunoblotting.



[1] Y. Shahali, J.-P. Sutra, G. Peltre, D. Charpin, H. Sénéchal, P. Poncet. IgE reactivity to Common cypress (*C. sempervirens*) pollen extracts: evidence for novel allergens. WAO Journal. 2010, 3, 229-234.

## RESULTS

Microscopic observation (Figure 1) confirms that the *Cupressus* pollen dry milling with CK14 beads during 3x30sec seems suited to generate small fragments of pollen.

The downstream analysis (results not shown) show that Minilys dry milling is very efficient to extract quickly high concentration of proteins. However, the protein extracted by dry milling were different from the protein extracted by overnight incubation in aqueous media (PBS).

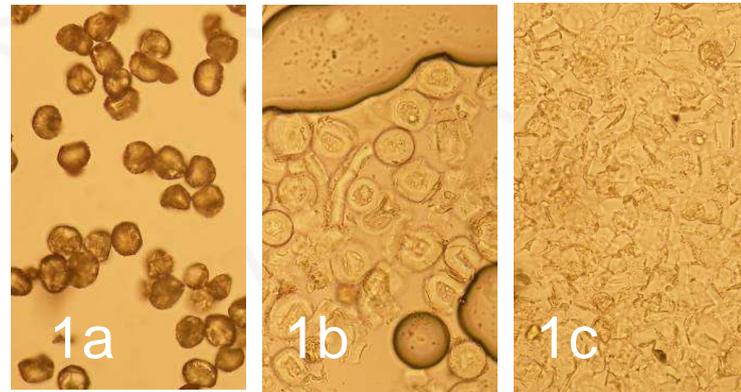


Figure 1: Microscopic observation (X40). Whole *Cupressus* pollen grain before grinding, dry observation (1a). *Cupressus* pollen grain after 1x30s dry grinding, observation in water (1b). *Cupressus* pollen grain after 3x30s dry grinding, observation in water (1c).

The dry milling is recommended in respect of cypress pollen due to his feature gelling in aqueous media.

Other pollen grains such as birch or grass pollen can be grind in liquid media.

## CONCLUSION

Minilys is successfully evaluated to extract high levels of proteins from cypress pollen by dry milling and generate fragments of smaller sizes for experiments and ultra structural analysis of immuno-reactivity.

The dry milling with **Minilys** could allow the revelation of different allergens patterns in the *Cupressus* pollen allergy and subsequently be applied to expand the panel of well-defined cypress pollen molecules for a more efficient allergen-based diagnosis and therapy.

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