Spectroscopic and Chemometric Profiling of Propolis and Cerumen Across Species and Regions.

Key differences identified using rPLS and STOCSY.

Jonas Vind[1], Søren Balling Engelsen[2], Henrik Munch Jørgensen[1], Julie Christine Antvorskov[3], Knud Josefsen[1], Violetta Aru[2].

Affiliations: [1]Department of Pathology, The Bartholin Institute, Copenhagen University Hospital, Copenhagen, Denmark, [2]Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark, [3]Department of Clinical Research, Steno Diabetes Center Copenhagen, Translational Type 1 Diabetes Research, Herlev, Denmark

Background

Propolis and cerumen are complex mixtures of beeswax, plant resins, and secretions, with composition influenced by geography and bee species. Understanding these differences is key to identifying antioxidant compounds.

Aim

To compare the composition and antioxidant-related compounds in Scandinavian (SC) and Australian (AU) *Apis mellifera* propolis and Australian *Tetragonula carbonaria* cerumen using phase-resolved ^{1}H NMR and recursive weighting partial least squares regression (rPLS).

Experimental

Samples were extracted using a modified Folch method to yield hydrophilic and hydrophobic phases for ^{1}H NMR analysis. Composition was assessed by PCA and wax structure by signal ratios. RSA data were modeled using full PLS and rPLS, and key signals were validated by standard addition.

Results

Scandinavian propolis had more aromatic compounds; Australian propolis had more carbohydrates; cerumen was rich in terpenoids. Wax content was highest in Australian propolis, but only *T. carbonaria* wax differed structurally. rPLS identified ferulic acid and p-coumaric acid as RSA-linked, with higher levels in Scandinavian samples.

Conclusion

^{1}H NMR combined with rPLS effectively revealed species- and region-specific differences in propolis and cerumen. Ferulic acid was identified as a key antioxidant in the aqueous phase, reflecting both compositional and RSA variation.

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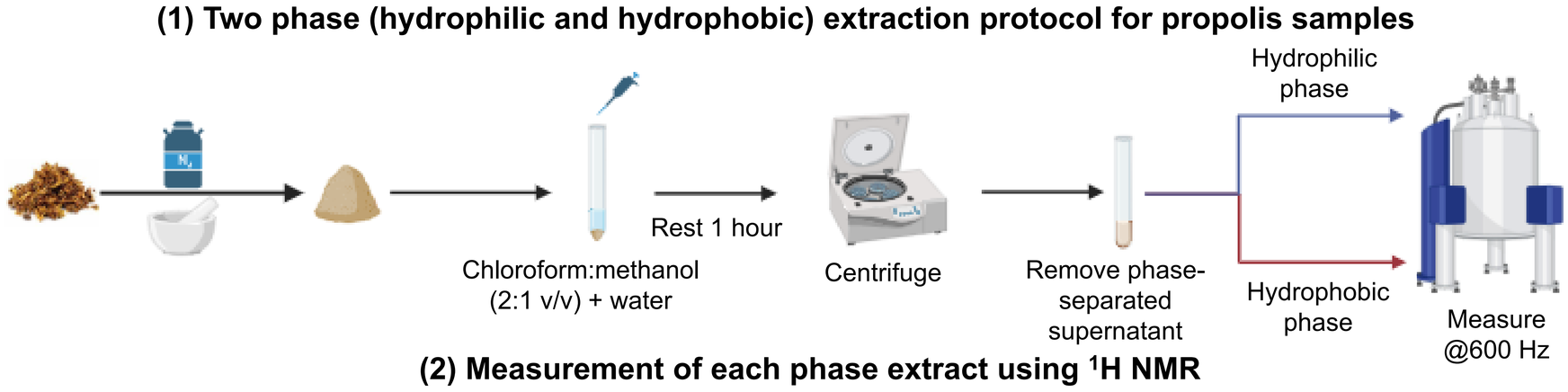
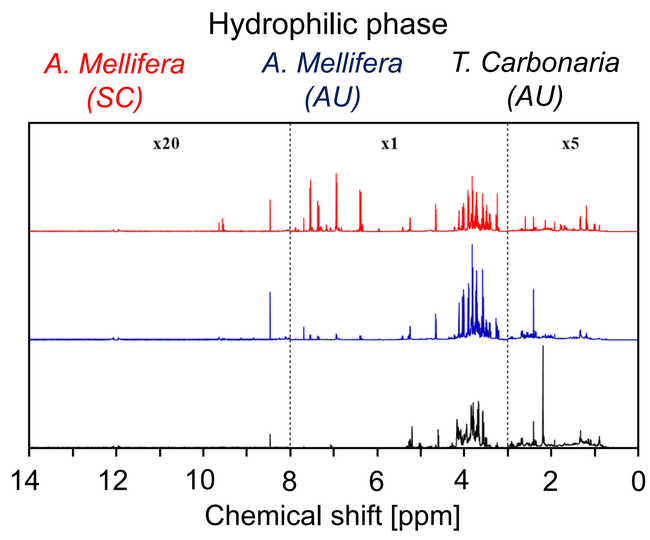
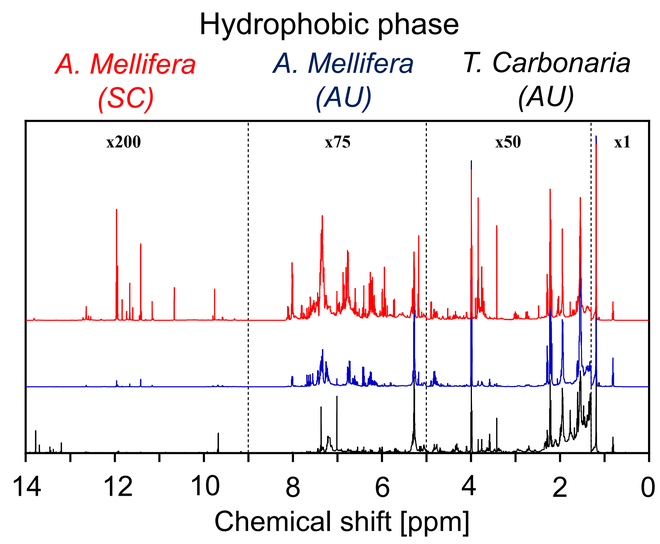
(1) Two phase (hydrophilic and hydrophobic) extraction protocol for propolis samples

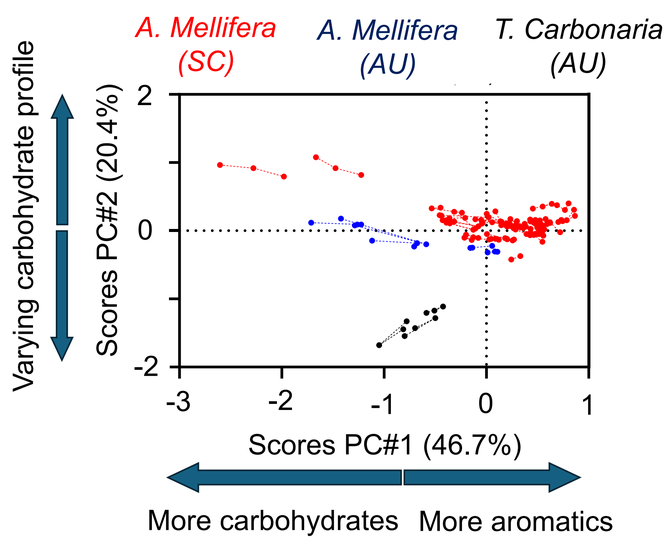


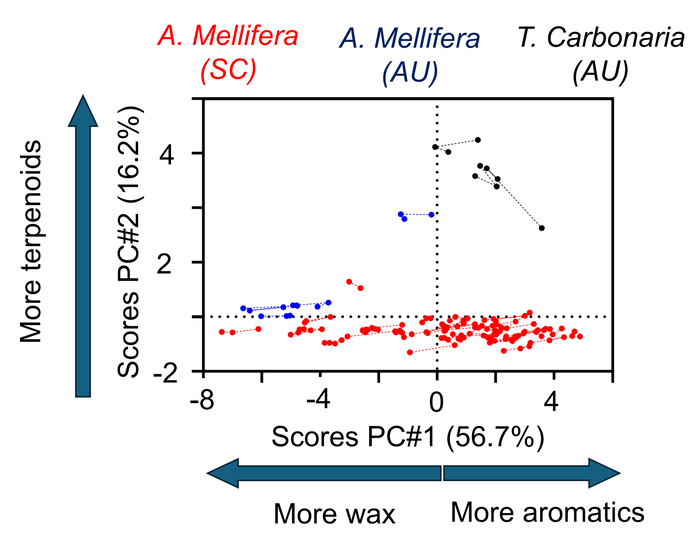
(2) Measurement of each phase extract using ^{1}H NMR





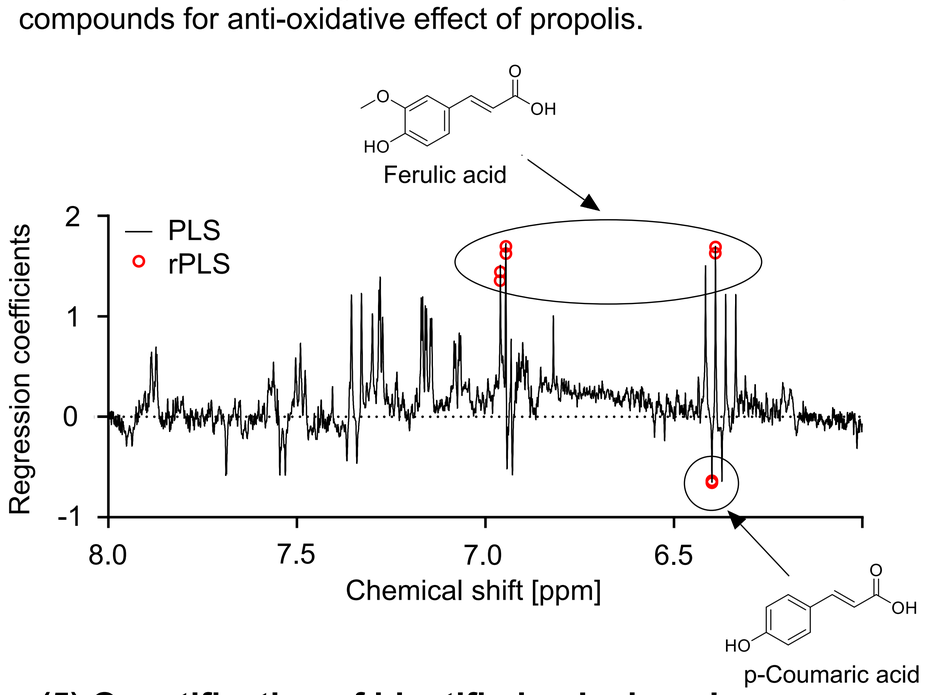
(3) Comparison of chemical composition using PCA





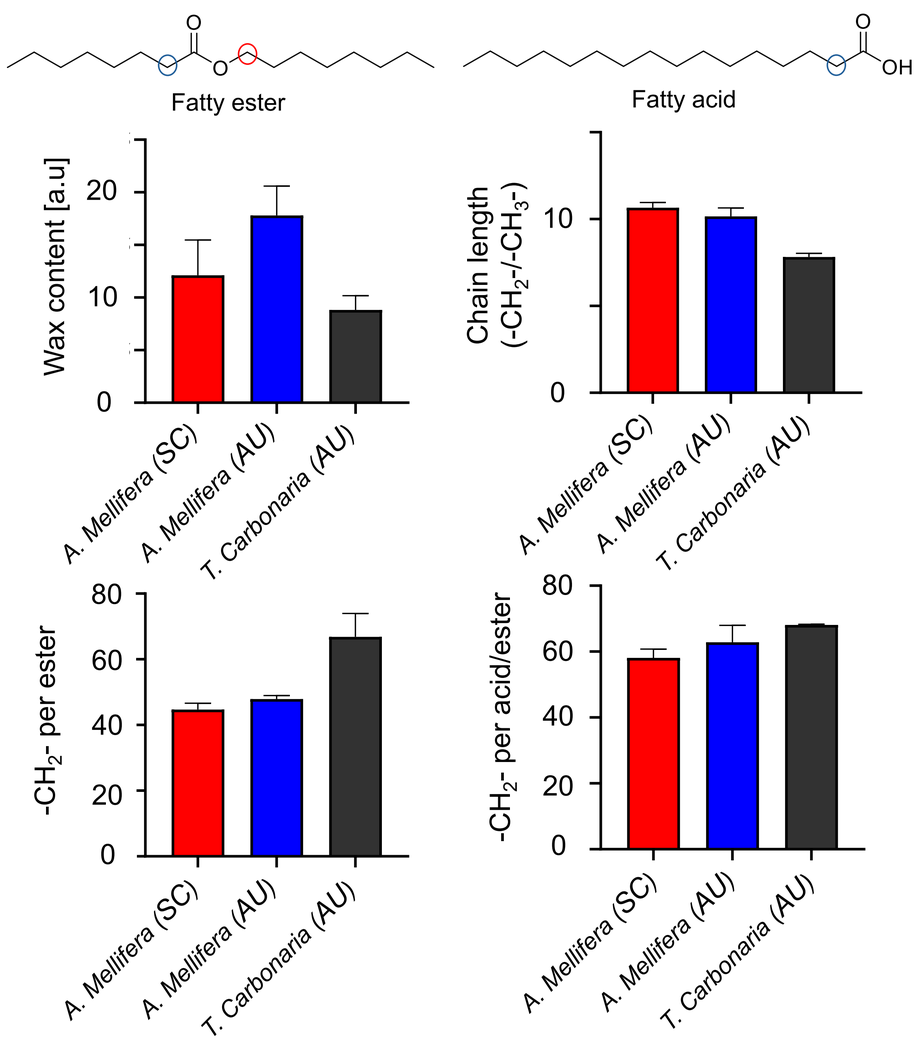
(4) Performance of rPLS to find most important anti-oxidative compounds in hydrophilic phase

A Full PLS and an rPLS model was created and compared. They performed similarly (**PLS: $RMSE_{CV}$ = 8.26%; rPLS: $RMSE_{CV}$ = 7.99 %**), and the selected signals in rPLS thus is indicative of important compounds for anti-oxidative effect of propolis.



(4) Comparison of beeswax in propolis and cerumen samples

Wax analysis of propolis and cerumen samples. Wax content was defined by CH_2 signal intensity in ^{1}H NMR; chain length as the CH_2/CH_3 ratio; chain length per fatty ester as the CH_2 to red-circled protons ratio; and chain length per fatty ester and acid as the CH_2 to blue-circled protons ratio.



(5) Quantification of identified polyphenols