Development of a new indicator of grape skin ripening in relation to *Botrytis cinerea* susceptibility

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Introduction

Grape maturity define the wine quality, evaluating sugar and acid contents, aromas evolution and phenolics extractability. Moreover, textural maturity may be used. It characterizes the grape skin maturity, corresponding to the cell wall evolution for a better extractability of phenolics and the increase of *Botrytis cinerea* susceptibility. Grape skin behaves as a hydrophobic barrier protecting against environmental factors such as UV, dehydration, physical injuries and pathogens. Grape skin morphology, cell wall composition and structure defines the grape skin capacity to resist to pathogen such as *Botrytis cinerea*[1]. The *Botrytis* rot constrains the Champagne elaboration by degrading the grape quality and the modification of the foaming properties[2]. The most common methods to control this rot are canopy management and the use of fungicides. In Bordeaux, IPP and IEP[3] have been developed to evaluate the textural maturity of grape skin in relation to *Botrytis cinerea* infection. Ripening process involves many changes in grape skin cell wall under the action of pectolytic enzymes such as polygalacturonases (PG) and pectin methylesterases (PME)[4] increasing *Botrytis cinerea* susceptibility. Referring to Bordeaux indicators (IEP and IPP), a combinate study of physical, biochemical, anatomical and molecular features could participate to the development of a new indicator of grape skin ripening in relation to *Botrytis cinerea* susceptibility, with the aim of reducing the use of fungicides and better manage the harvest date.

Material and method

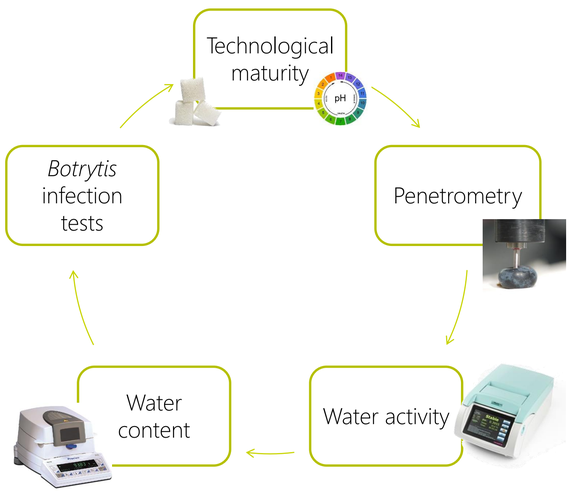
CHARDONNAY
less susceptible

PINOT NOIR
more susceptible

Vintage 2019

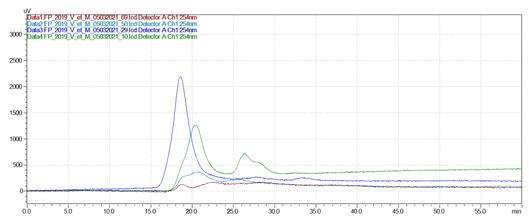
36th stage of development from Eichhorn and Lorenz scale

SKIN CHARACTERISTICS



BIOCHEMICAL FEATURES

High Performance
Size Exclusion Chromatography



SKIN ANATOMY

Transmission
Electronic microscope

MOLECULAR ASSAY

Gene expression

Enzyme activity

Results

SKIN CHARACTERISTICS

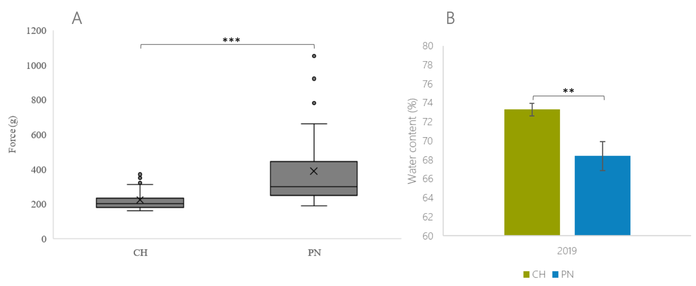


Figure 1. Penetrometry (A) and water content (%) (B) in grape skin from Chardonnay (CH) and Pinot noir (PN)
*** p-value < 0,001; ** p-value < 0,01

- Pinot noir skins required more force to penetrate the skin than Chardonnay skins
- Water content is higher in Chardonnay than Pinot noir skins

SKIN ANATOMY

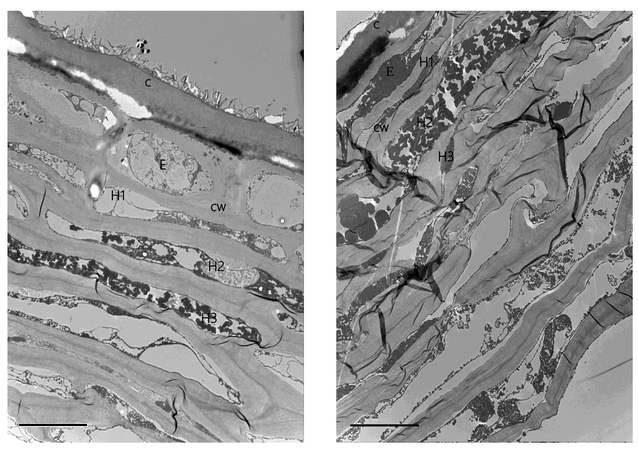


Figure 2. Ultrastructure observation of grape skin from Chardonnay and Pinot noir
Bar = 10 µm; cuticle (c); cell wall (cw); epidermis (E); hypodermis 1 (H1); hypodermis 2 (H2); hypodermis 3 (H3)

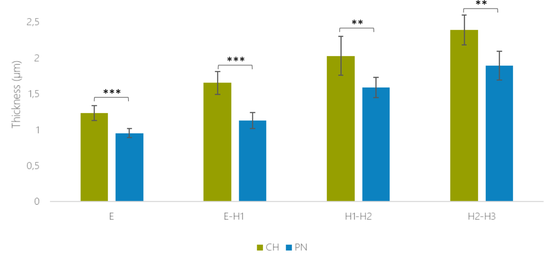


Figure 3. Cell wall tickness (µm) for the different cell layers from Chardonnay (CH) and Pinot noir (PN) skin
Mean ± confidence interval at α=0,05. E : epidermis ; H1 : hypodermis 1 ; H2 : hypodermis 2 ; H3 : hypodermis 3.
*** p-value < 0,001; ** p-value < 0,01

- Different cell layers observed for both cultivars : cuticle (c), epidermis (E) and hypodermis (H)
- Ultrastructure less complex in Chardonnay skins
- Thinner cell walls for Pinot noir compared to Chardonnay

BIOCHEMICAL FEATURES

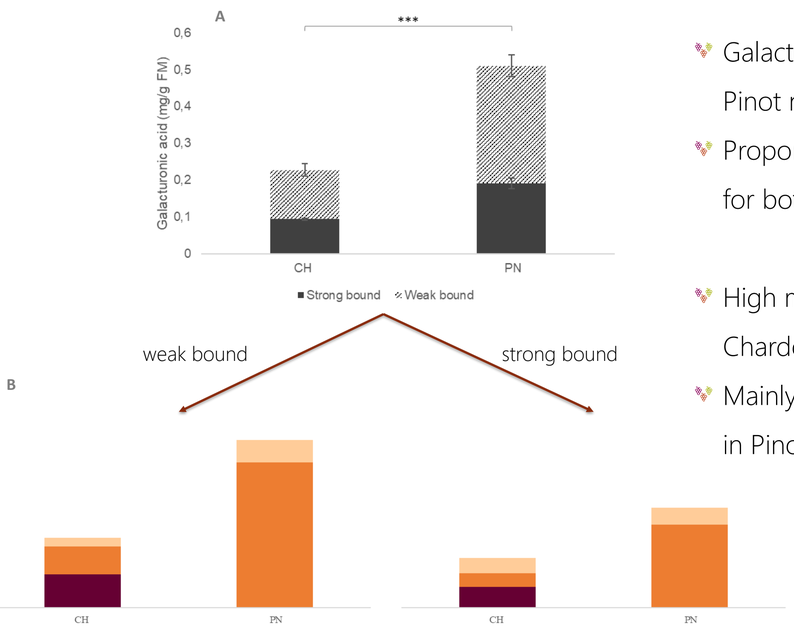


Figure 4. Galacturonic acid content (A) and polysaccharides distribution (B) according to their molecular mass by type of bonds in grape skin cell walls from Chardonnay (CH) and Pinot noir (PN)

- Galacturonic acid content significantly higher in Pinot noir cell walls
- Proportion of strong bond and weak bond similar for both cultivars (40/60).
- High molecular mass polysaccharides in Chardonnay cell walls (>380 kDa)
- Mainly medium molecular mass polysaccharides in Pinot noir cell walls (380 kDa to 47 kDa)

MOLECULAR ASSAY

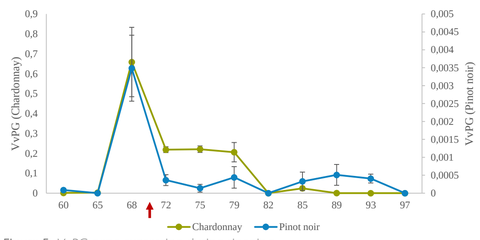


Figure 5. *VvPG* gene expression during ripening

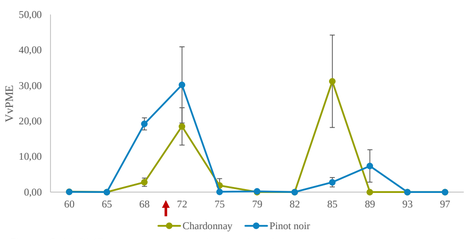


Figure 6. *VvPME* gene expression during ripening

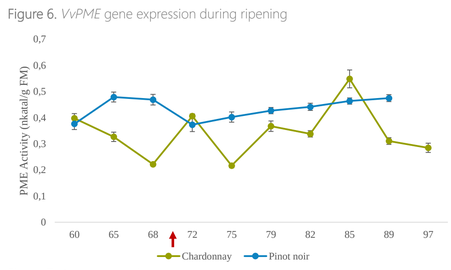


Figure 7. PME activity during ripening

- Same profile observed for both cultivar agreeing with literature
- *VvPG* and *VvPME* expressed all along ripening
- *VvPG* present a high expression period for Chardonnay skins
- *VvPME* gene expression similar for both cultivars
- PME activity detected all along ripening
- PME activity remains stable during ripening for Pinot noir compared to Chardonnay skins

Conclusion

The aim of this study was to understand the different mechanisms involved in the grape skin ripening process at veraison, of two cultivars from Champagne region to develop a new indicator of grape skin ripening. By a combination of physical, biochemical, structural and molecular parameters, we were able to define a profile of grape skin for the two cultivars at veraison related to their susceptibility to *Botrytis cinerea*. According to our results, Pinot noir seems more susceptible by its cell wall composition and thickness. Its skin appears thinner than Chardonnay one and presents more soluble polysaccharides appetible to *Botrytis cinerea*. Moreover, Pinot noir skins are more fragilized due to degrading enzymes action at veraison. Also, physical analyses (penetrometry, water content and water activity (data not shown) could be use to evaluate the grape skin fragility related to *Botrytis cinerea* susceptibility. As already developed in Bordeaux (IPP and IEP), these indicators could be used to better manage the harvest date in Champagne.

[1] Mlikota Gabler *et al.*, 2003
[2] Marchal *et al.*, 2001
[3] Brummell, 2006
[4] Deytieux-Belleau, *et al.*, 2009

Acknowledgements
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