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Bernhard Kuster  
TU München  
kuster@tum.de

Simone Lemeer  
TU München  
simone.lemeer@tum.de

Katrin Marcus  
Ruhr-Universität Bochum  
katrin.marcus@rub.de

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MPI für biophysikalische Chemie  
henning.urlaub@mpibpc.mpg.de



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# Identification and Quantification of Proteins in *Malus domestica* Affected by Bitter Pit

Michael Krawitzky<sup>1,2,3</sup>, Jesus Val<sup>2</sup>, Didier Chevret<sup>1</sup>, Céline Henry<sup>1</sup>, Rosa Oria<sup>3</sup>, Veronique Monnet<sup>1</sup>

<sup>1</sup> UMR 1319 MICALIS, Plateforme Protéomique de Paris Sud Ouest (PAPPSO), Institut National de la Recherche Agronomique (INRA), Jouy-en-Josas (France)

<sup>2</sup> Estación Experimental de Aula Dei - Consejo Superior de Investigaciones Científicas (EEAD - CSIC), Zaragoza (España)

<sup>3</sup> Universidad de Zaragoza, Facultad de Veterinaria, C/ Miguel Servet, 177 Zaragoza (España)



## Introduction

Bitter pit is a physiological disorder that occurs in apple, pear, and quince and has been associated with calcium uptake or lack thereof. Although bitter pit has been studied for over a century, there is still not enough knowledge about bitter pit and why there are no completely effective preventive treatments to reduce fruit loss. More than 70 million tonnes of apple fruit were globally produced in 2010 (FAOSTAT), with an estimated commercial value of over \$29 million. According to group data (unpublished), upwards of 30% of apple fruit can be affected with bitter pit. In a previous group publication (Val et al. 2006), it was conjectured after SDS-PAGE that an unknown 18 kDa protein might contribute to bitter pit.



Figure 1. Bitter pit spots in *Malus domestica*.

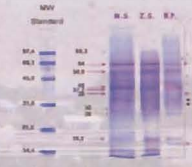


Figure 2. Bitter pit SDS-PAGE analysis.

## Objective

The objective of the present study was to get more extensive data on the proteomic changes associated to bitter pit using mass spectrometry-based proteomics for protein identification and label free relative quantification.

## Materials and Methods

Samples (*Malus domestica* 'Reinette gris du Canada' and 'Golden Smoothee') were collected late-August 2011, near Zaragoza (Aragón), Spain. Following phenol extraction, protein (10µg) were allowed to run on SDS-PAGE, trypsin digested, and digestion products were analyzed on a Q-Exactive mass spectrometer (Thermo Scientific).

Protein identification was performed querying MS/MS data against the apple genome database (+30,000 proteins, direct protein (annotated) sequence type, apple genome database (<http://genomics.research.iasma.it>), together with an in-house contaminant database, using the X!Tandem pipeline software (<http://pappso.inra.fr/>). Proteins identified with at least two unique peptides and a log (E-value) lower than 4 ( $10^{-4}$ ) were validated.

## Results and Discussion

More than 200 proteins were identified in the range of 12-24 kDa, using spectral counting for relative quantification. The protein range was chosen based on previous group studies investigating low molecular weight heat shock proteins (sHSPs). ANOVA was performed to determine if the factor bitter pit could be considered significant. Results show 34 proteins with significantly different values ( $P < 0.01$ ) between bitter pit and healthy samples. We focused on these 34 proteins that varied significantly between the two conditions (bitter pit vs. healthy) being over/under expressed.

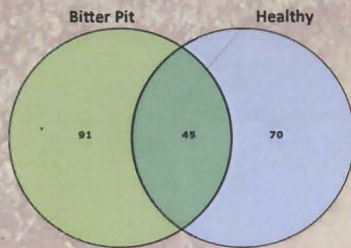


Figure 4. Venn Diagram distribution of bitter pit and healthy *Malus domestica* proteins identified between 12-24 kDa.

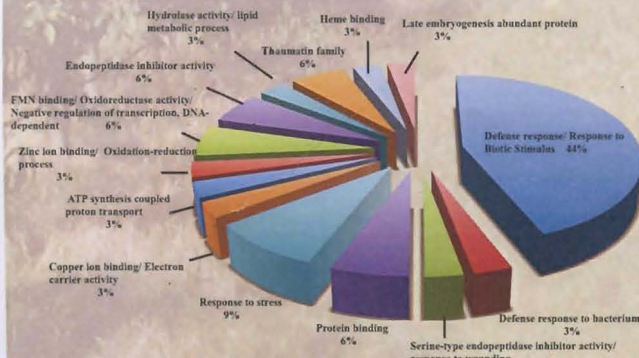


Figure 6. Identified Gene Ontology (GO) in Bitter Pit Affected *Malus domestica*.

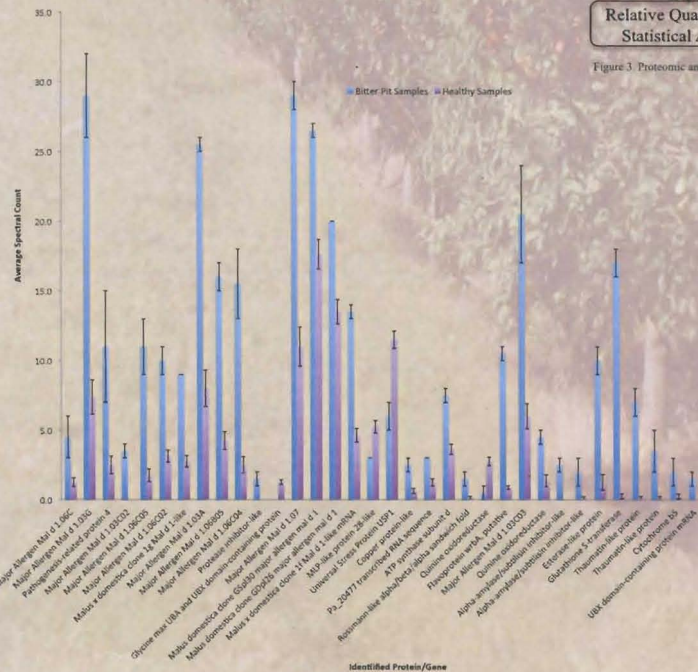


Figure 5. Spectral count average comparisons between bitter pit and healthy *Malus domestica* proteins, in the 12-24 kDa range.

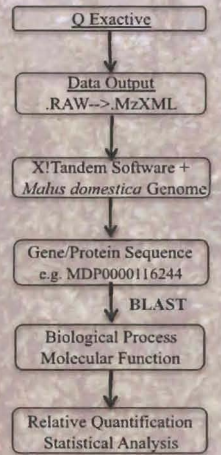


Figure 3. Proteomic analysis flowchart.

## Conclusions

Several proteins identified near or at 18 kDa, are related to *Malus domestica* response to stress, desiccation, and increased protein binding. Considerable differences were found in proteins related to pathogenesis (defense response/ response to biotic stimulus) between bitter pit and healthy samples, proteins previous identified as allergens belonging to the Bet v 1 family.

## Acknowledgement

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- Val, J., M. A. Gracia, A. Blanco, E. Monge, and M. Perez. 2006. Polypeptide pattern of apple tissues affected by calcium-related physiopathologies. Food Science and Technology International 12(5):417-422.



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## Identification and quantification of proteins in *Malus domestica* affected by bitter pit

Michael Krawitzky<sup>1</sup>, Didier Chevret<sup>2</sup>, Céline Henry<sup>2</sup>, Rosa Oriá<sup>3</sup>, Veronique Monnet<sup>2</sup>, Jesus Val<sup>4</sup>

<sup>1</sup> UMR 1319 MICALIS, Plateforme Protéomique de Paris Sud Ouest (PAPPSO), (INRA), Jouy-en-Josas (France).

Estación Experimental de Aula Dei Consejo Superior de Investigaciones Científicas (EEAD - CSIC). Zaragoza (España). Universidad de Zaragoza, Facultad de

<sup>2</sup> UMR 1319 MICALIS, Plateforme Protéomique de Paris Sud Ouest (PAPPSO), Institut National de la Recherche Agronomique (INRA), Jouy-en-Josas (France)

<sup>3</sup> Universidad de Zaragoza, Facultad de Veterinaria, C/ Miguel Servet, 177 Zaragoza (España)

<sup>4</sup> Estación Experimental de Aula Dei - Consejo Superior de Investigaciones Científicas (EEAD - CSIC), Zaragoza (España)

Bitter pit is a physiological disorder that occurs in apple, pear, and quince and has been associated with calcium uptake or lack thereof. Although bitter pit has been studied for over a century, there is still not enough knowledge about bitter pit and why there are no completely effective preventive treatments to reduce fruit loss. In a previous group publication (Val et al. 2006), it was conjectured after SDS-PAGE that an unknown 18 kDa protein might contribute to bitter pit. The objective of the present study was to identify this 18 kDa protein and get more extensive data on the proteomic changes associated to bitter pit using the latest mass spectrometry-based proteomics.

Healthy and bitter pit fruit samples (*Malus domestica* 'Reinette gris du Canada' and *Malus domestica* 'Golden Smoothee') were collected near Zaragoza (Aragón, Spain). Following phenol extraction, ten µg protein were allowed to run on SDS-PAGE, trypsin digested, and digestion products were analyzed on a Q-Exactive mass spectrometer (Thermo Scientific). Proteins were identified with X!Tandem pipeline software (<http://pappso.inra.fr/>) and relative quantification was performed by spectral counting.

More than two hundred proteins were identified in the range of 12-24 kDa. We focused on 35 proteins that varied significantly between the two conditions (bitter pit vs. healthy) being over/under expressed. There were 26 and 22 bitter pit proteins ('Reinette gris du Canada' and 'Smoothee Golden Delicious,' respectively) detected with at least 50% greater abundance when compared to their respective healthy counterparts. Among these proteins, 14 found in both cultivars, were identified as Pathogenesis-related protein Bet v I, a major allergen found in trees within the order Fagales. In both apple cultivars, 2 proteins were identified as thaumatin-like protein (TLP), a group of proteins responsible for several fruit allergies. Glutathione S-transferase, linked to protein binding and heat shock transcriptional factors (Hsfs) in *Malus*, was abundantly detected in bitter pit samples for both cultivars.

Several proteins identified near or at 18-kDa, are related to *Malus domestica*' response to stress, desiccation, and increased protein binding. Considerable differences were found in allergen concentrations between bitter pit and healthy samples, suggesting an increased allergen risk for consumers who ingest bitter pit affected fruit.