Discovering natural bioactive peptides in cheese with mass spectrometry

Randall Robinson,[1] Florine Citerne,[1] David Dallas,[3] and Daniela Barile[1,2]
[1]Department of Food Science and Technology [2]Foods for Health Institute, University of California, Davis, Davis, CA
[3]School of Biological and Population Health Sciences, Oregon State University, Corvallis, OR 97331

Introduction

Proteolysis can produce peptides with beneficial biological functions, such as anti-bacterial,[1] immunomodulatory,[2] and anti-hypertensive[3] activities. Most dairy products contain naturally-occurring peptides, many of which have yet to be characterized for their sequences, cleavage sites, and functional abilities. Identifying the functions of peptides existing naturally in foods may be important in understanding their biological impact.

The objective of this project is to use mass spectrometry to identify the peptides that are present in a variety of cheeses and characterize some of their functional attributes. The cheeses selected for this study contain organisms which are intentionally added for flavor development and contribute to quality, ripening, and proteolysis. Thus far, peptides have been identified in cheddar, Stilton (Britain), Taleggio (Italy), and Mimolette (France) cheeses. These peptides will be annotated for function with a functional search program. Peptides with valuable functions may show promise for therapeutic or translational applications.

Methods

Peptides were extracted in duplicate as shown in Figure 1 and analyzed on a Thermo Orbitrap Q Exactive Plus mass spectrometer. Precursor ions were selected for fragmentation through data dependent acquisition (DDA), and peptides were identified from fragment spectra with X!Tandem software using a nonspecific enzymatic cleavage pattern. The bovine proteome was downloaded from Uniprot and used by X!Tandem as a reference for peptide identification. Maximum allowable m/z errors were set at 20 ppm for precursor and fragment ions.

Table 1. Description of cheeses.

Cheese	Aging time	Proteolytic factors
Mimolette	6-8 weeks	Bacteria, rennet, mites
Stilton Blue	6-12 weeks	Rennet, *Penicillium roqueforti*
Taleggio	4-5 weeks	Bacteria, rennet, molds
Cheddar	< 8 weeks	Bacteria, rennet

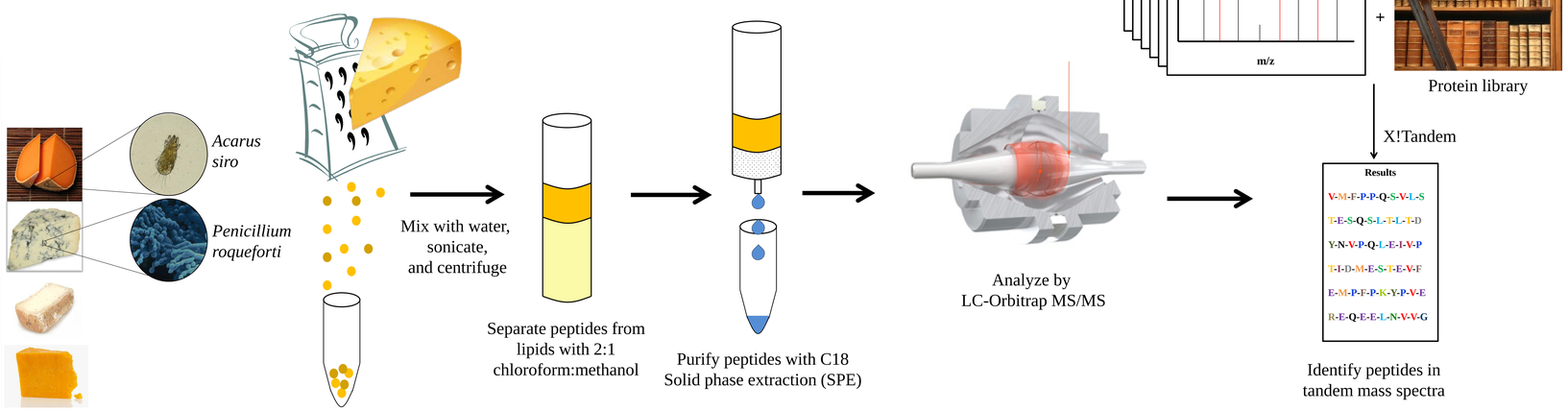
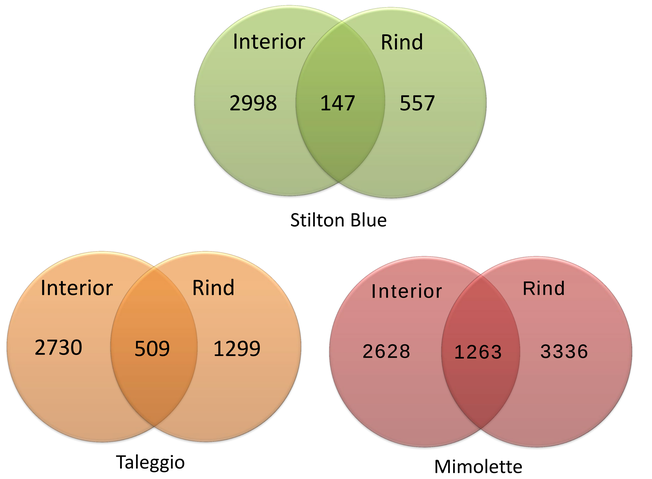


Figure 1. Workflow for peptide extraction, analysis, and identification.

Results

- In total, an automated search of the tandem spectra revealed over 1000 peptides in each cheese. In all cases, the majority of peptides were derived from caseins.
- Interestingly, the greatest numbers of peptides were found in Mimolette. Since Mimolette is made from the same type of milk as the other cheeses and ages for a similar length of time, the greater degree of proteolysis may be a result of the specific starter cultures used or a result of digestion by the *Acarus siro* mites that normally live on the cheese rind.
- The number of peptides identified in the rind of Stilton Blue was much lower than that of the interior. It is possible that proteolysis in the rind proceeds to such an extent that the milk proteins are degraded into very small units (i.e. dipeptides and tripeptides), which are generally not detected by the automated peptide search. Additionally, the cheese wheels are usually punctured with stainless steel rods to promote mold growth on the interior (eventually giving rise to the characteristic blue veins).
- Differences in peptide sequences between the interior and rind of each cheese may indicate that different factors influence proteolysis in each region.
- Future work will include an enzyme analysis to identify the enzymes potentially responsible for the production of the identified peptides (i.e. enzymes from *Acarus siro* mites).
- The peptides identified in this experiment will also be annotated for function using an in-house program which matches peptides to known functional sequences.



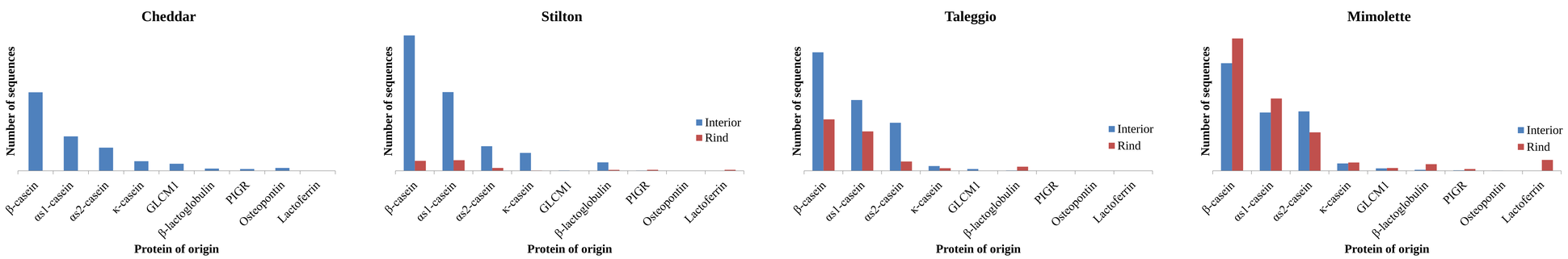


Figure 2. Identified peptides grouped by protein of origin.

Conclusion

Over 1000 peptides sequences were identified in each cheese using an enzyme-nonspecific search. To date, this study represents the most extensive examination of the naturally-occurring peptides in these dairy products. Different factors appear to influence protein digestion in the rind and interior of Stilton Blue, Taleggio, and Mimolette.

Further analysis will identify the enzymes responsible for proteolysis in the rind and interior of each cheese, and the homology of the identified peptides to known functional sequences will be assessed. Peptides with relevant biological functions may be valuable for therapeutic applications.

References

1. Dallas, D.C.; Guerrero, A.; Khaldi, N.; Castillo, P.A.; Martin, W.F.; Smilowitz, J.T.; Bevins, C.L.; Barile, D.; German, J.B.; Lebrilla, C.B. *J. Proteome Res.* **2013,** *12*, 2295-2304.

2. Malinowski, M.K.; Klempt, M.; Clawin-Radecker, I.; Lorenzen, P.Chr.; Meisel, H. *Food Chem.* **2014,** *165*, 129-133.

3. Stuknytė, M.; Cattaneo, S.; Masotti, F.; De Noni, I. *Food Chem.* **2015,** *168*, 27-33.

Acknowledgements

Randall Robinson was awarded the 2013 Jastro-Shields research grant and would like to acknowledge the support the grant has provided in funding this project.

The authors would also like to thank the entire Barile Lab for their suggestions and support.