

# APPLICATION NOTE

## #53

## LET IT BRIE: Using the non-destructive SepQuant dropletProbe™ sampling system to analyze the free amino acid content of various cheeses

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### Application & Background

The methods of cheese-making and ripening are complex and often likened more to an art than a science.<sup>1</sup> However, as more has been discovered about the microbial communities and biochemical processes that produce the distinct flavor profiles and textures of various cheeses, cheesemongers and affineurs have been able to apply new analytical techniques to their cheese aging and quality control practices.<sup>1</sup> Casein proteins are an important energy source for the microbial communities that drive the complex process of surface-ripening of various types of cheese.<sup>2</sup> The process of proteolysis involves cleavage of these caseins into smaller peptides, then free amino acids, and culminates in amino acid catabolism, which is one of the primary biochemical reactions that produces flavor in cheese.<sup>2</sup> Here, we aimed to use the SepQuant dropletProbe, an innovative liquid extraction surface analysis sampling system, coupled to high performance liquid chromatography-mass spectrometry (HPLC-MS) to profile the free amino acid content of 12 different cheeses.

### Experimental

#### Sample Preparation

All cheese was purchased at Trader Joe's and stored at 20°C until sectioning. Samples were taken from the 12 different cheeses using a razor blade and allowed to dry overnight at room temperature on the benchtop. Remaining liquid on the surface of the cheese was blotted off each sample individually using KimWipes. Samples were mounted onto glass slides using double-sided tape.



Figure 1. Some of the commercially-available cheeses analyzed in this study.

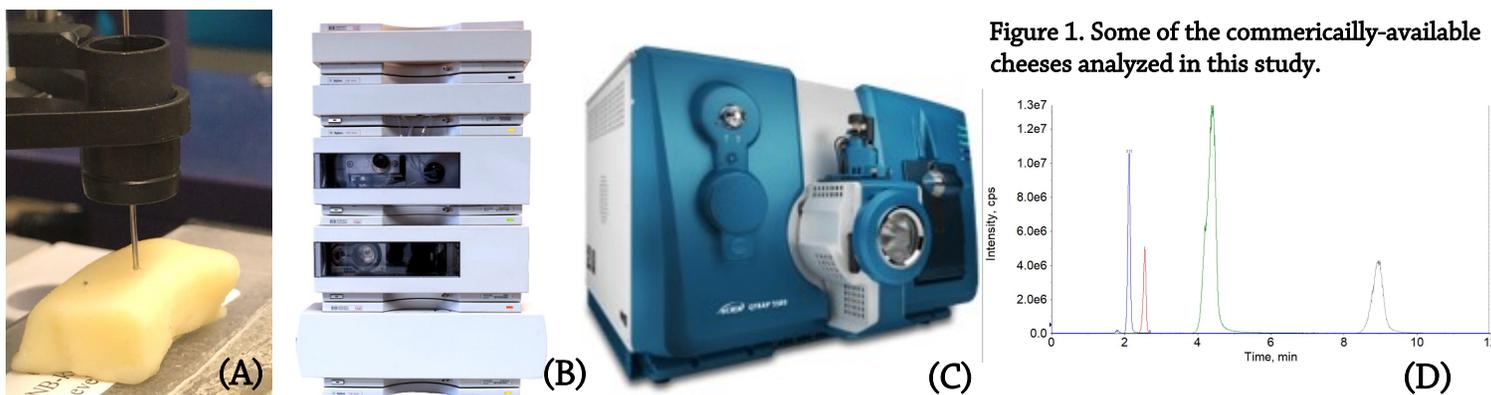
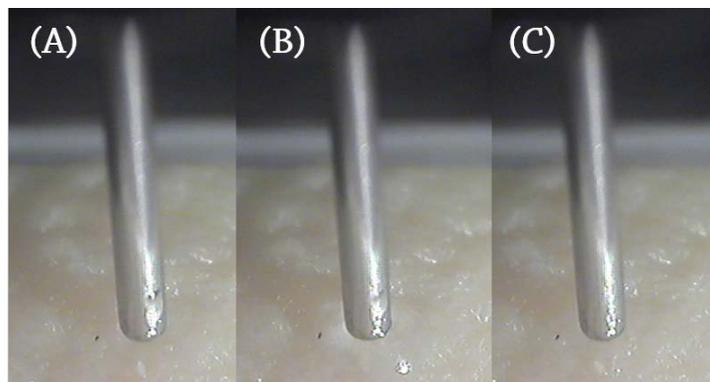


Figure 2. Overview of experimental workflow: (A) All cheese sections were sampled via the SepQuant® dropletProbe™, (B) the droplets were separated using an Agilent 1100 HPLC system with an in-line Waters PAH, C-18 column, (C) data were collected using an electrospray AB Sciex QTRAP® 5500, and (D) analyzed using MRM triggered MS/MS (data shown: MRM extracted chromatograms for proline (blue), valine (red), leucine (green), and phenylalanine (gray)).

# APPLICATION NOTE

## Surface Sampling using the SepQuant® dropletProbe™

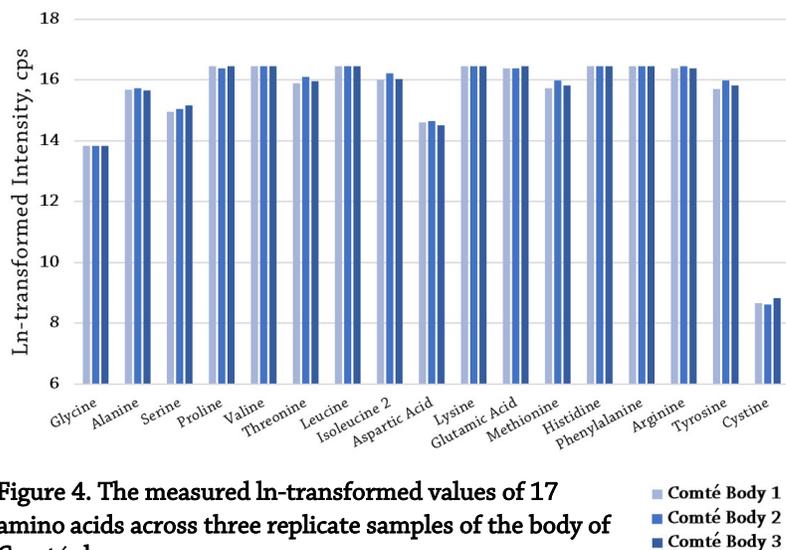
Surface sampling was performed using 1 µL droplets of H<sub>2</sub>O dispensed from a 100 µL syringe filled with 10 µL H<sub>2</sub>O using the SeqQuant® dropletProbe™. Two extraction cycles were implemented at each sample location. Samples were set up to run in batches. Three quality photos were acquired for each sampling event to provide a quality control measure during the automated, unsupervised data acquisition (Figure 3).



**Figure 3. Examples of quality control photos obtained for every sampling event by the SeqQuant® dropletProbe™:** (A) Before droplet disbursement, (B) during surface sampling, and (C) upon retraction of the droplet.

## HPLC/MS-MS Analysis

The total volume in the syringe of 10 µL was injected into Agilent 1100 HPLC system with an in-line Waters PAH, C-18, 5 µm, 2x150 mm column. Analytes were isocratically separated using 100/0.1 (v/v) H<sub>2</sub>O/formic acid at a 200 µL/min flow rate for 10 minutes. Data were collected on an AB Sciex QTRAP® 5500 system using positive mode electrospray ionization. Optimized MRM transitions for the 17 amino acids were adopted from a prior publication.<sup>3</sup> Total analysis time was 12 minutes for each sample. All data were natural log-transformed to conform to a near-normal distribution.



**Figure 4. The measured ln-transformed values of 17 amino acids across three replicate samples of the body of Comté cheese.**

Statistical tests, including analysis of variance (ANOVA) and two-sided t-tests were used to analyze the transformed data.

## Results

### Reproducibility of SepQuant dropletProbe™ Data

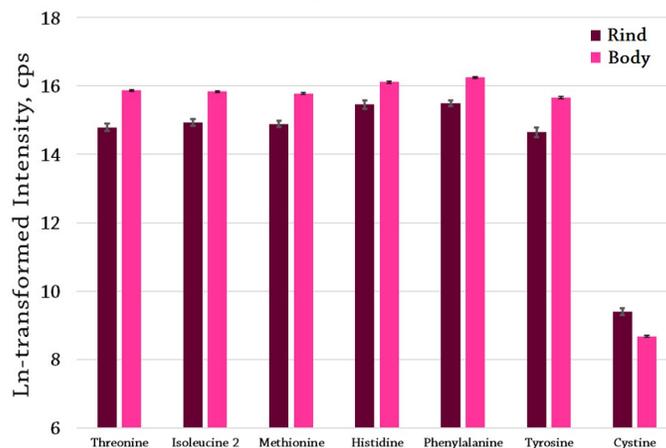
In order to assess reproducibility of the SepQuant® dropletProbe™ sampling technique, biological replicate samples (i.e. from the same region of the same cheese) were taken for multiple samples. Results from a single-factor ANOVA indicated no significant difference (p-value = 0.99) between the means across any of the amino acids for these repeated samples (Figure 4).

### Levels of Amino Acids are Higher in the Body versus Rind Region of the Cheeses

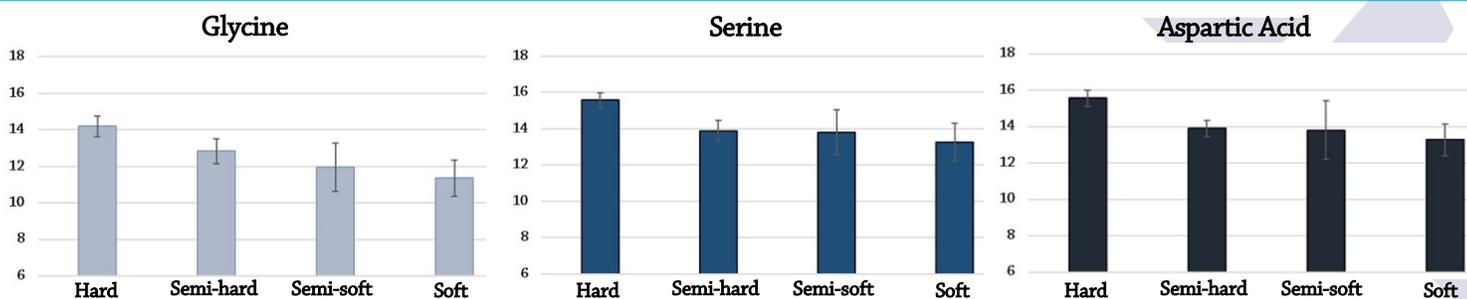
Results from two-sided t-tests indicated that levels of six amino acids were present at significantly higher levels (p-value < 0.05) in areas of the body of cheeses versus the rind. One amino acid, cystine was present in greater quantities in the rind versus the body of these cheeses. were used to compare the relative abundance of the 17 measured amino acids in the rind versus the body regions of the cheeses (Figure 5).

### Levels of Amino Acids Vary in Relation to Cheese Hardness

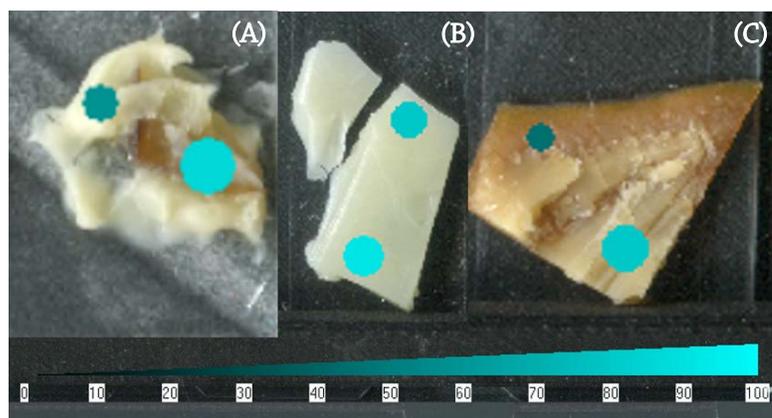
Using the classifications given on the cheese labels, cheeses were classified into four categories of (1) Hard, (2) Semi-Hard, (3) Semi-soft, and (4) Soft. One-way ANOVA tests were used to test for differences in the means of each of the 17 measured amino acids among these four groups. A Bonferroni correction was applied to the resulting p-values to account for multiple tests and 99% confidence intervals were calculated. Glycine, serine, and aspartic acid tended to be present at higher levels in harder cheeses (Figure 6).



**Figure 5. The measured averages of ln-transformed values of the 7 amino acids that were significantly different in the rind versus the body region of the cheeses.**



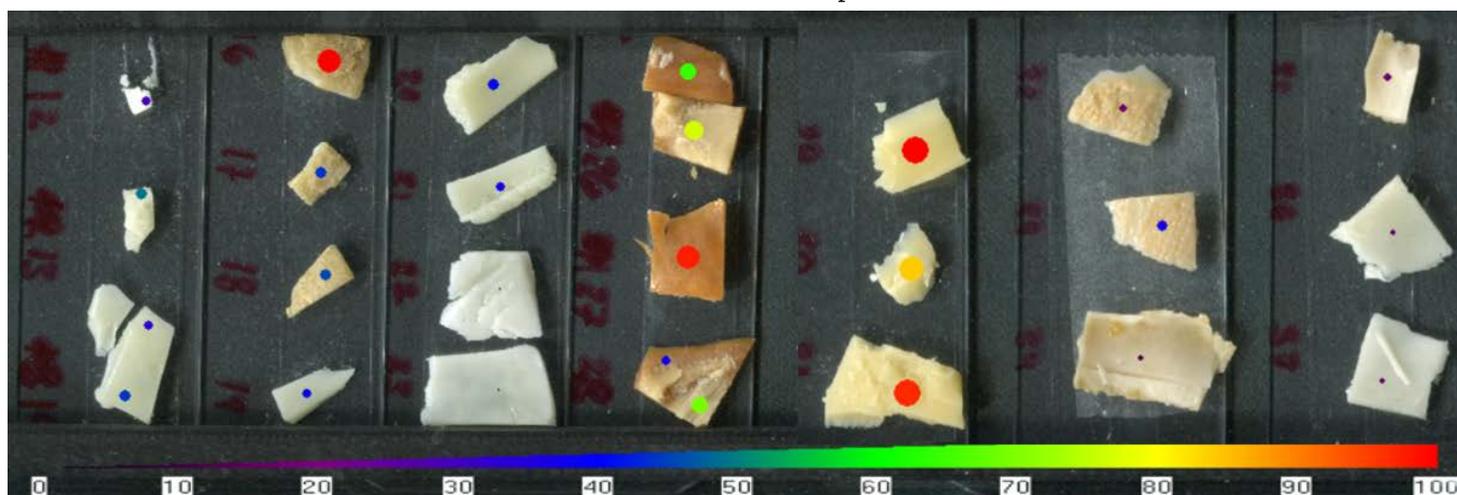
**Figure 6. Significant differences between cheeses grouped by hardness were detected for 3 of the 17 measured amino acids: glycine, serine, and aspartic acid. These three amino acids tended to be present at higher levels in harder cheeses.**



**Figure 7. The SepQuant® dropletProbe™ software interface allowed for easy visualization of intrasample variability as it related to spatial features of the sample. (A) Proline was more abundant in the mushroom of the triple cream brie cheese with mushrooms than in the body of the cheese. (B) Valine was more abundant in the air-exposed hole of the Emmentaler cheese than the inside. (C) Leucine was more in the inner rind than the outer rind of the gouda cheese.**

## Conclusions

The amino acid profile between and within different cheese samples varies considerably. In contrast to typical LC-MS sample preparation of sample homogenization, surface sampling via the SepQuant® dropletProbe™ allowed us to query different locations within a single sample to observe heterogeneity across the surface of various cheeses (Figure 8). In three of the samples, 2 locations representing different biological conditions were measured in separate sampling events (Figure 7). The SepQuant® dropletProbe™ also allowed for unsupervised, automated sampling of many cheese specimens with easy visualization of relative quantification of all 17 amino acids. In this study, we demonstrated that the SepQuant® dropletProbe™ sampling system is a sensitive, efficient, and non-destructive method to characterize the free amino acid content of many different types of cheese. Future research could aim to apply this same concept to a variety of different known flavor compounds in cheese and other foodstuffs and to monitor the production of flavor compounds from the same sample in situ over the course of time.



**Figure 8. The heatmap depiction of relative aspartic acid levels in different cheese samples. Aspartic acid has previously been indicated to be one of the amino acids that primarily composes casein proteins.<sup>1</sup>**

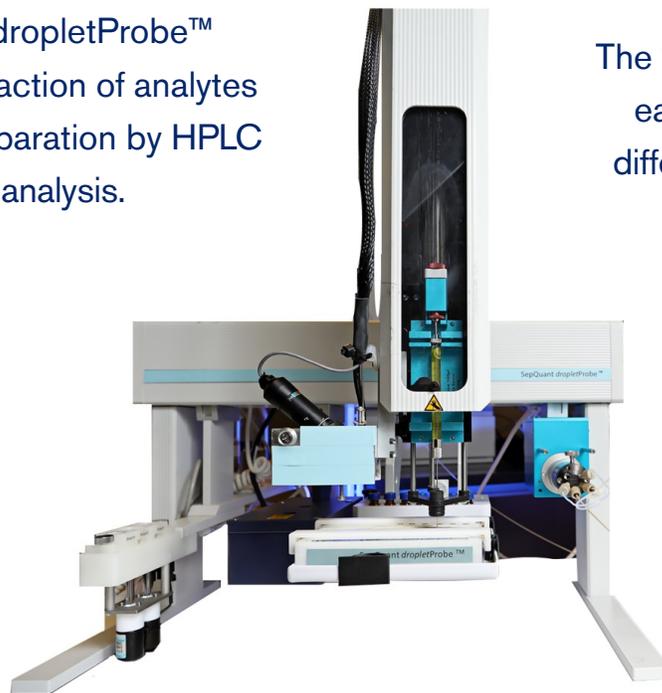
## References

- <sup>1</sup>Johnson ME. A 100-Year Review: Cheese production and quality. *J Dairy Sci.* 2017;100(12):9952-9965. doi:10.3168/jds.2017-12979
- <sup>2</sup>Dugat-Bony E, Straub C, Teissandier A, et al. Overview of a surface-ripened cheese community functioning by meta-omics analyses. *PLoS One.* 2015;10(4):1-25. doi:10.1371/journal.pone.0124360
- <sup>3</sup>Lane CS. Rapid LC-MS/MS Analysis of Free Amino Acids in Extracellular Matrix. *Biomarkers Omi.* 2015;(RUO-MKT-02-3777-A):17-19.

# SepQuant *dropletProbe*™

Non-destructive, *in situ* Sampling (NDIS) system

The SepQuant® *dropletProbe*™ provides *in situ* extraction of analytes coupled to in-line separation by HPLC prior to MS analysis.



The SepQuant® *dropletProbe*™ easily integrates with many different mass spectrometers.

## Key Characteristics of the SepQuant® *dropletProbe*™

- ❖ Acquisition of optical images of the surface for point-and-click selection of locations to be sampled
- ❖ Liquid junction droplets from 0.5 to 5 µL
- ❖ Laser sensor-to-surface technology for precise and automated positioning of the liquid interface for samples of disparate heights
- ❖ Spatial resolution as low as 700 µm
- ❖ <1 minute sampling time
- ❖ Oversampling available
- ❖ Heatmap generation
- ❖ Method can be integrated into existing LC/MS workflow

## Applications of the SepQuant® *dropletProbe*™

- ❖ Drugs and metabolites, antibody–drug conjugate catabolites and macromolecules (proteins and oligonucleotides) from thin tissue sections
- ❖ Drugs from tissue biopsy
- ❖ Metabolites from fungal cultures
- ❖ Small molecules from plant tissues (e.g. capsaicin from peppers, caffeine from coffee beans)
- ❖ Food science applications

SepQuant *dropletProbe*™ is available worldwide exclusively from HTX Technologies, LLC.

To request further information contact:

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