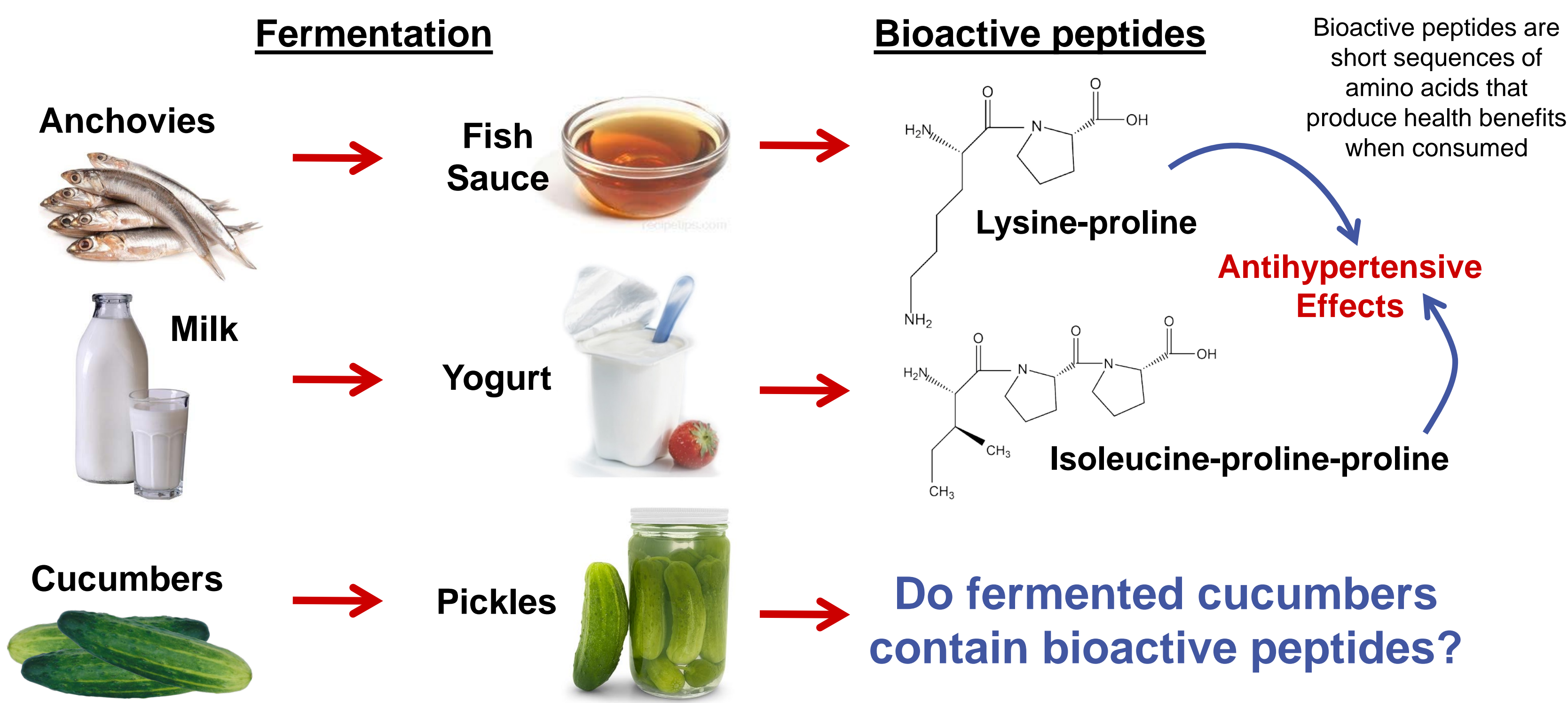


## Introduction

Foods fermented with lactic acid bacteria are known to contain health promoting compounds, including bioactive peptides



**Objective:** Use direct analysis infrared matrix-assisted laser desorption/ionization (IR-MALDESI) mass spectrometry (MS) to identify peptides in fermented cucumbers

## Methods

- 1 Compile internal database of food-derived bioactive peptides from the literature
- 2 Prepare fermented and acidified cucumbers in triplicate

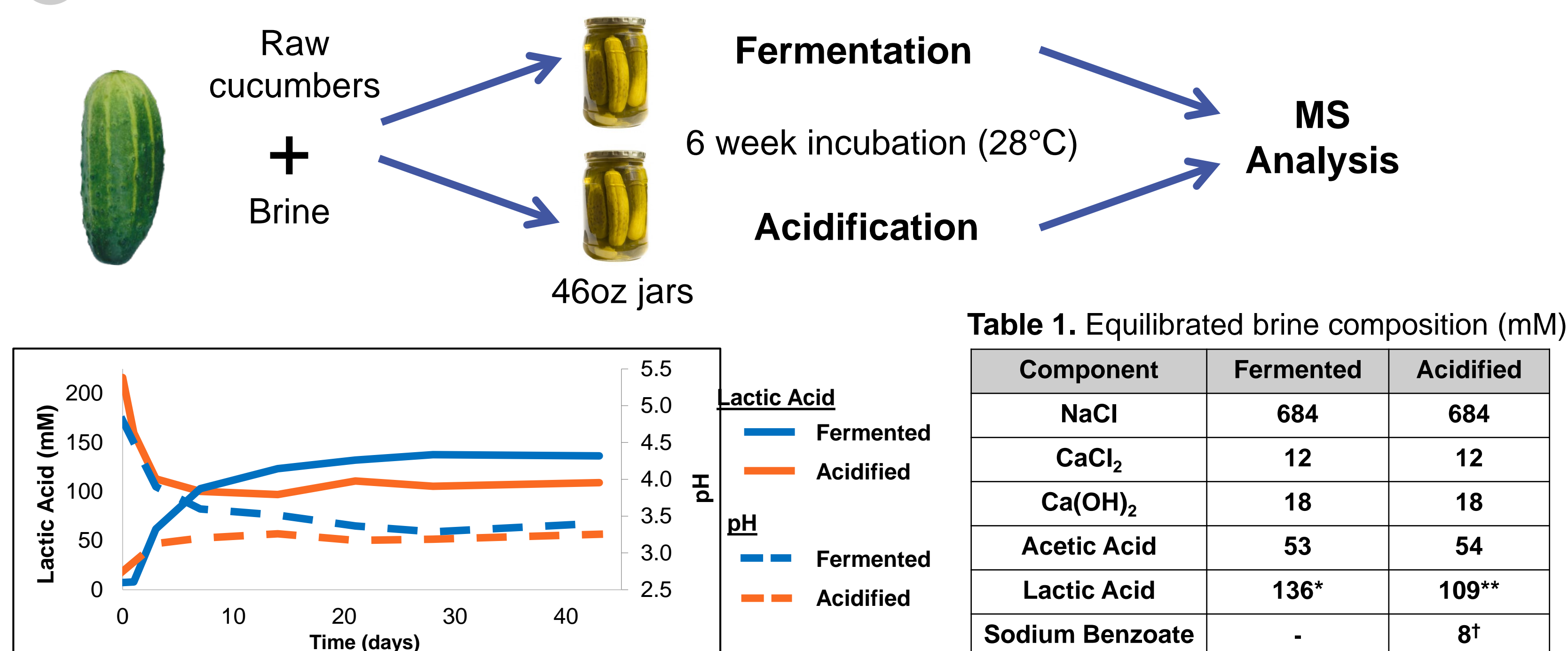
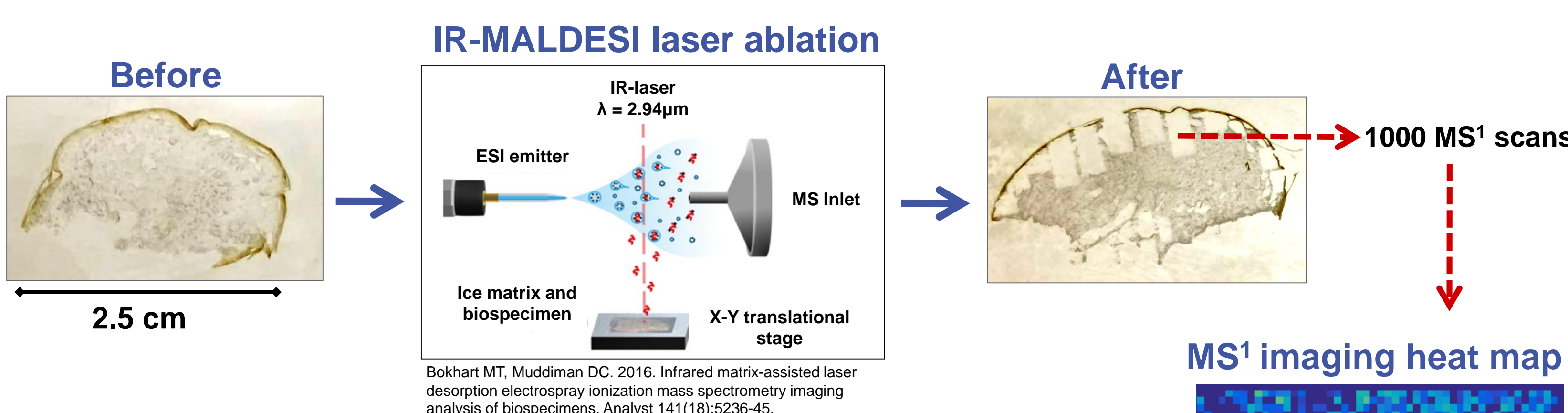


Figure 1. Lactic acid and pH over time

- 3 Directly detect small molecules in 100µm thick cucumber slices



- 4 Identify putative bioactive peptide matches by MS<sup>1</sup> imaging
- 5 Confirm matches by IR-MALDESI MS/MS (MS<sup>2</sup>) spectral analysis

## Results

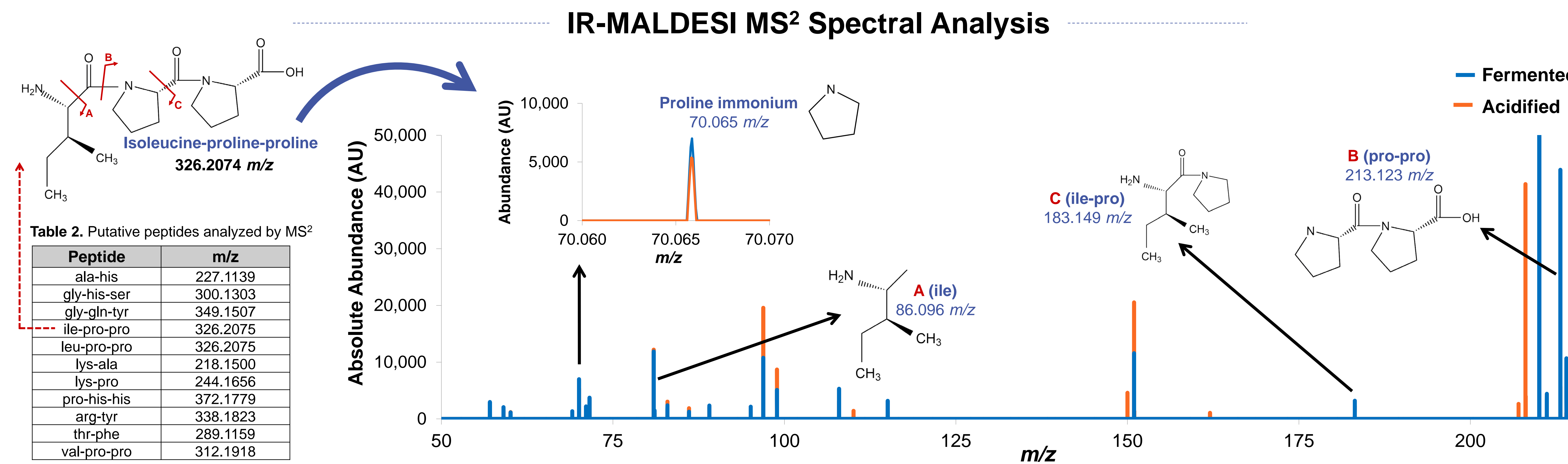
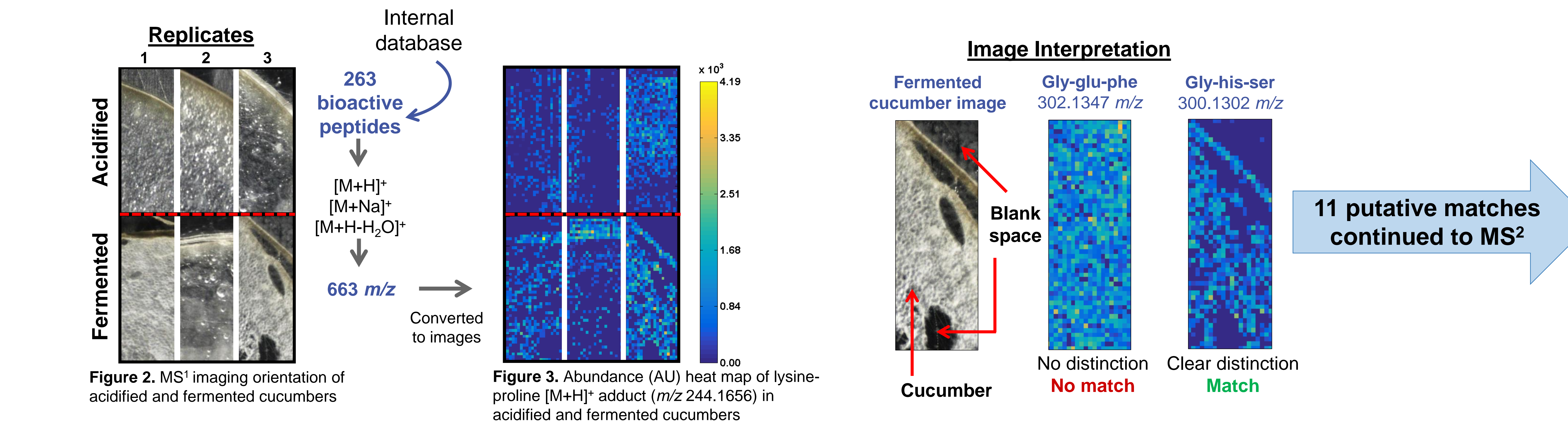
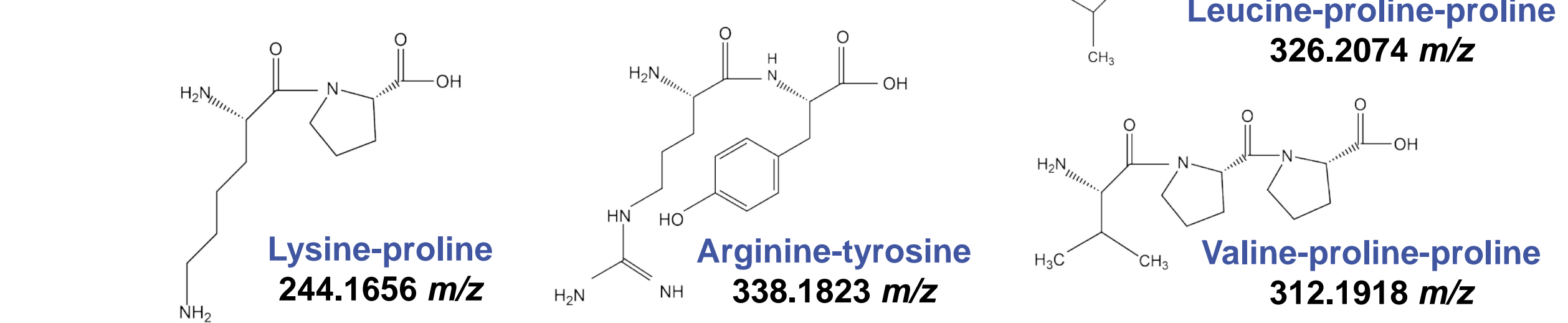


Figure 4. Average MS<sup>2</sup> spectra of the putative match for isoleucine-proline-proline<sup>‡</sup> at 326.2m/z ± 1Da with 140,000 nominal resolving power

### 4 bioactive peptides confirmed by MS<sup>2</sup>

- All confirmed bioactive peptides were previously documented in foods and possess angiotensin-I converting enzyme (ACE) inhibitory activity
- ACE inhibition lowers blood pressure



**Table 3. Bioactive peptides and characteristic fragments confirmed by MS<sup>2</sup> in fermented and acidified cucumbers**

| Peptide (collision energy)             | Fragments identified (m/z (abundance)) |                |                |                |                |               |
|--|--|----------------|----------------|----------------|----------------|---------------|
|  | Fermented                              |                |                | Acidified      |                |               |
| Ile/Leu-pro-pro <sup>‡</sup> (19.5 eV) | 70.065 (6500)                          | 86.096 (2420)  | 116.071 (5930) | 70.065 (5450)  | 86.096 (1870)  | 116.071 (465) |
| Lys-pro (24.4 eV)                      | 70.065 (25000)                         | 84.081 (66100) | 86.096 (1410)  | 70.065 (18700) | 84.081 (23600) | 86.096 (6460) |
| Arg-tyr (27.1 eV)                      | 70.065 (4760)                          | 136.076 (1060) | 175.119 (3390) | 70.065 (4340)  | 136.076 (932)  | 175.119 (247) |
| Val-pro-pro (18.7 eV)                  | 70.065 (9740)                          | 116.071 (1120) | 169.133 (6150) | 70.065 (276)   | 169.133 (146)  | 213.123 (147) |

<sup>‡</sup> Isoleucine-proline-proline and leucine-proline-proline have the same m/z value and fragmentation patterns. Either or both of these peptides may be present in the samples.

## Conclusions

- Direct analysis IR-MALDESI effectively ionized peptides from a high salt matrix with minimal sample preparation
- Four ACE inhibitory bioactive peptides were discovered in fermented cucumbers
- Bioactive peptides were documented for the first time in a fermented vegetable