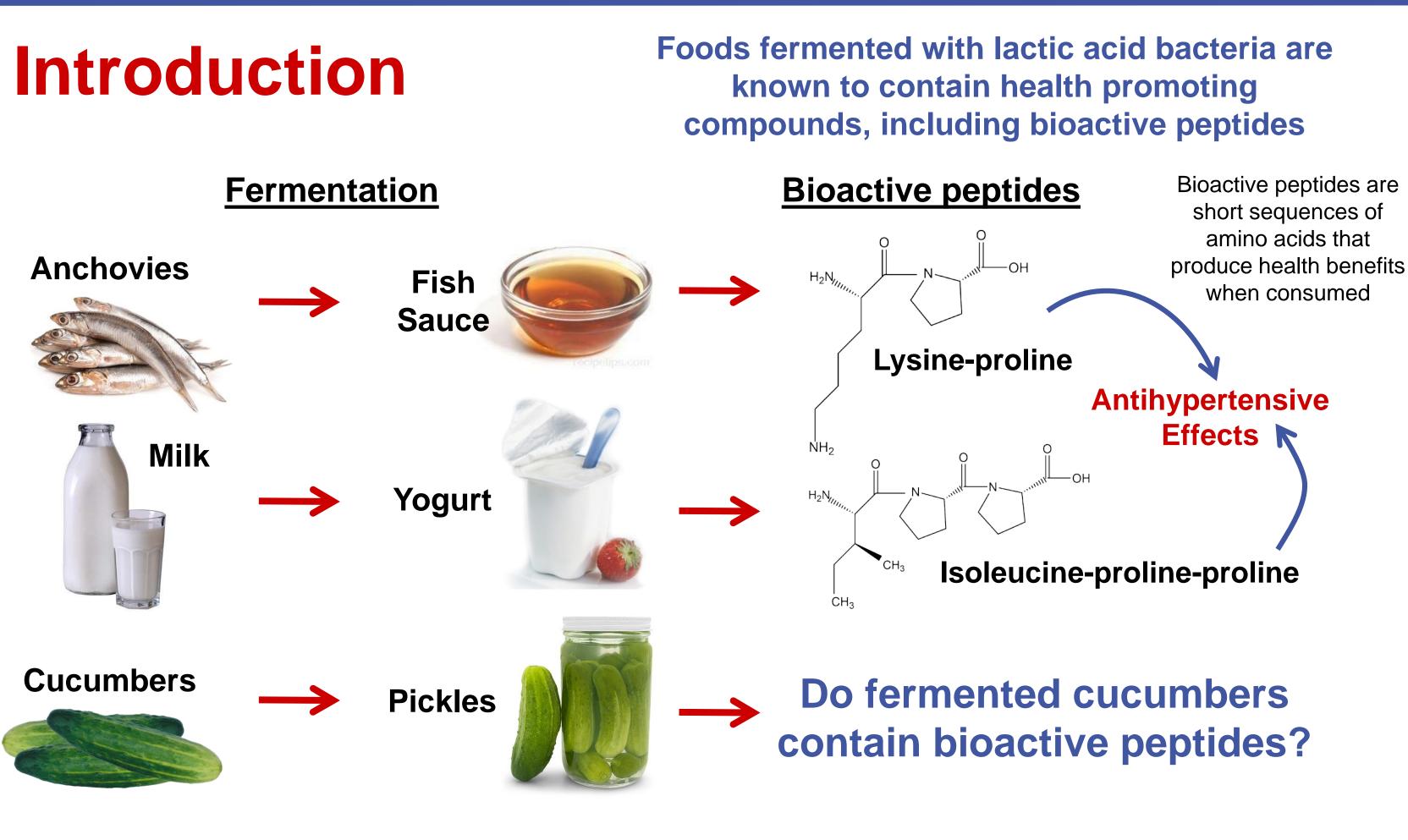


# Discovery of bioactive peptides in fermented cucumbers by direct analysis IR-MALDESI mass spectrometry

Jennifer Fideler<sup>1, 2</sup>, Måns Ekelöf<sup>3</sup>, David C. Muddiman<sup>3</sup>, Suzanne D. Johanningsmeier<sup>2, 1</sup> <sup>1</sup>Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC; <sup>2</sup>Food Science Research Unit, USDA Agricultural Research Service, Raleigh, NC; <sup>3</sup>Department of Chemistry, North Carolina State University, Raleigh, NC; <sup>4</sup>Food Science Research Unit, USDA Agricultural Research Service, Raleigh, NC; <sup>4</sup>Food Science Research Unit, USDA Agricultural Research Unit, USDA Agricultural Research Unit, USDA Agricultural Research Service, Raleigh, NC; <sup>4</sup>Food Science Research Unit, USDA Agricultural Research Unit, USDA Agric

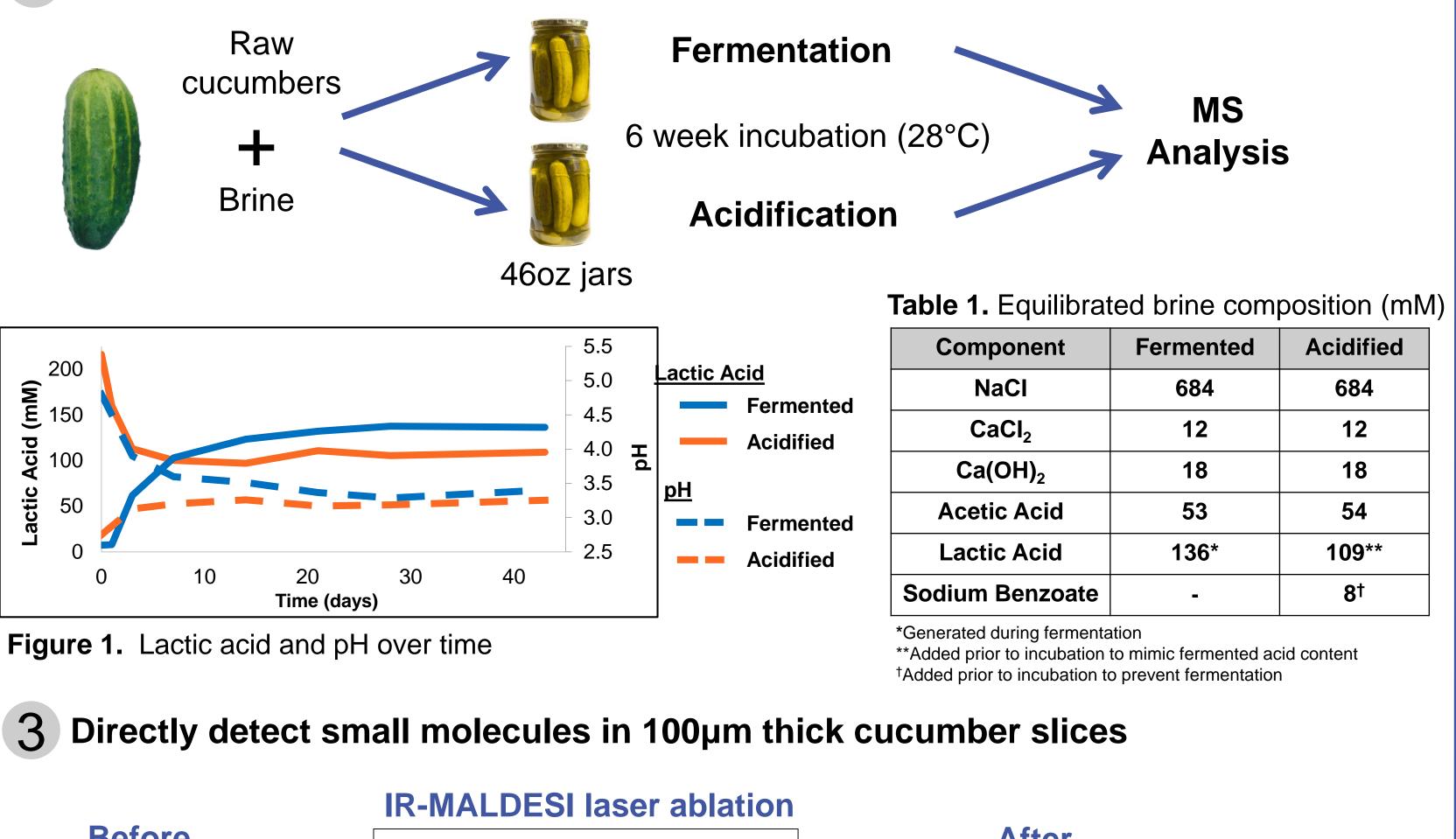


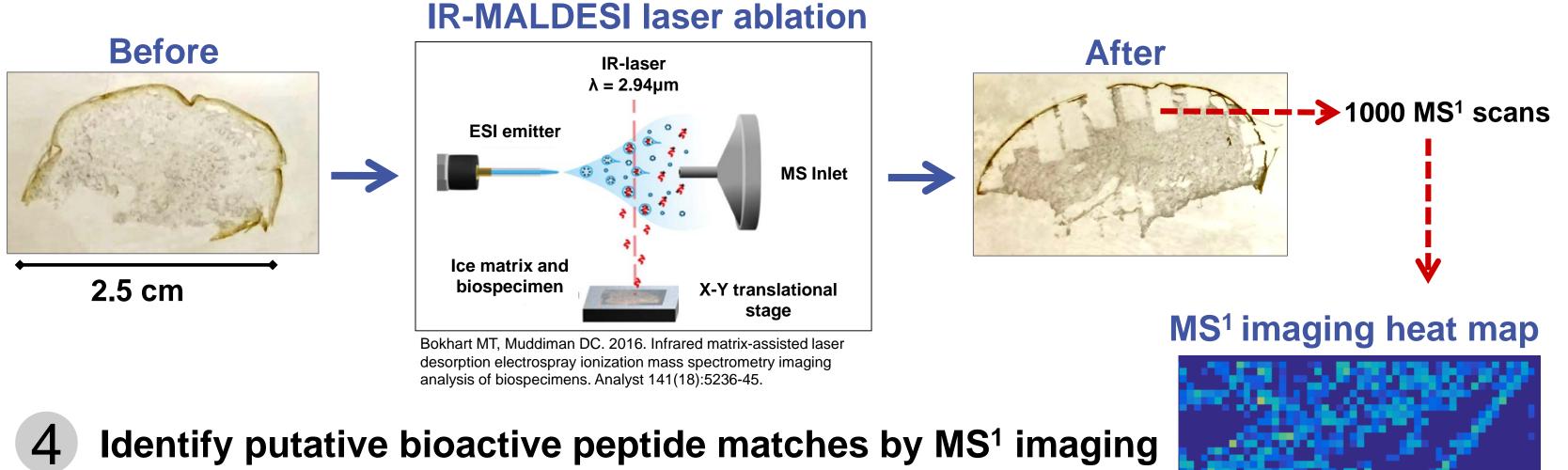
**Objective:** Use direct analysis infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry (MS) to identify peptides in fermented cucumbers

# Methods

Compile internal database of food-derived bioactive peptides from the literature

**2** Prepare fermented and acidified cucumbers in triplicate

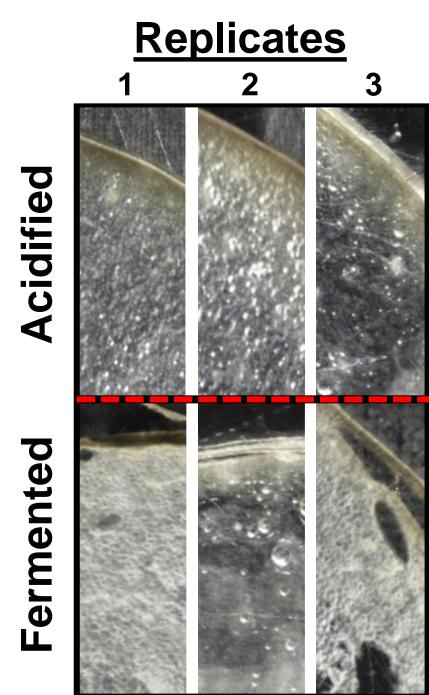


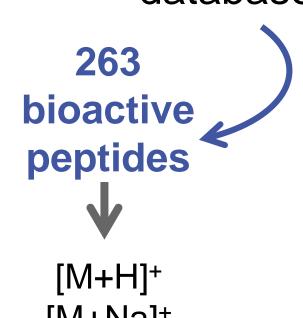


**5** Confirm matches by IR-MALDESI MS/MS (MS<sup>2</sup>) spectral analysis

nt	Fermented	Acidified		
	684	684		
	12	12		
2	18	18		
id	53	54		
id	136*	109**		
zoate	-	8†		

# Results





[M+Na]+  $[M+H-H_2O]^+$ 

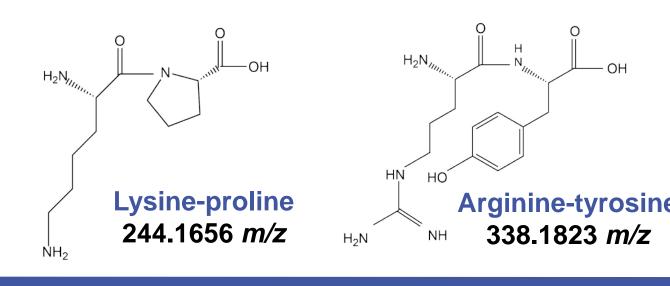
Figure 2. MS<sup>1</sup> imaging orientation of acidified and fermented cucumbers

$H_2N_{H_2N_{H_2}} \rightarrow H_2N_{H_2} \rightarrow H_2 \rightarrow H$					
	Peptide	m/z	Abundance (AU		
	ala-his	227.1139	pl d		
	alv-his-ser	300 1303	A		

gly-his-ser	300.1303		
gly-gln-tyr	349.1507		
ile-pro-pro	326.2075		
leu-pro-pro	326.2075		
lys-ala	218.1500		
lys-pro	244.1656		
pro-his-his	372.1779		
arg-tyr	338.1823		
thr-phe	289.1159		
val-pro-pro	312.1918		

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

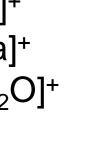
- All confirmed bioactive peptides were previously documented in foods and possess angiotensin-I converting enzyme (ACE) inhibitory activity
- ACE inhibition lowers blood pressure



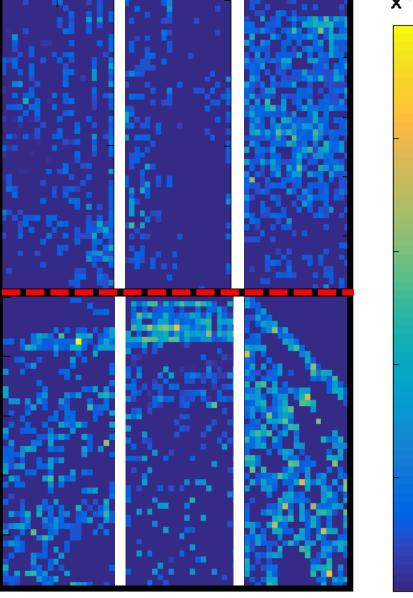
Conclusions

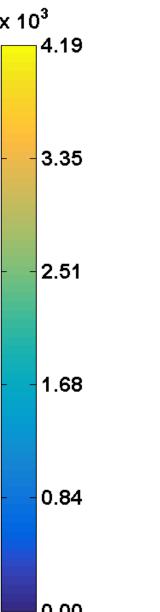


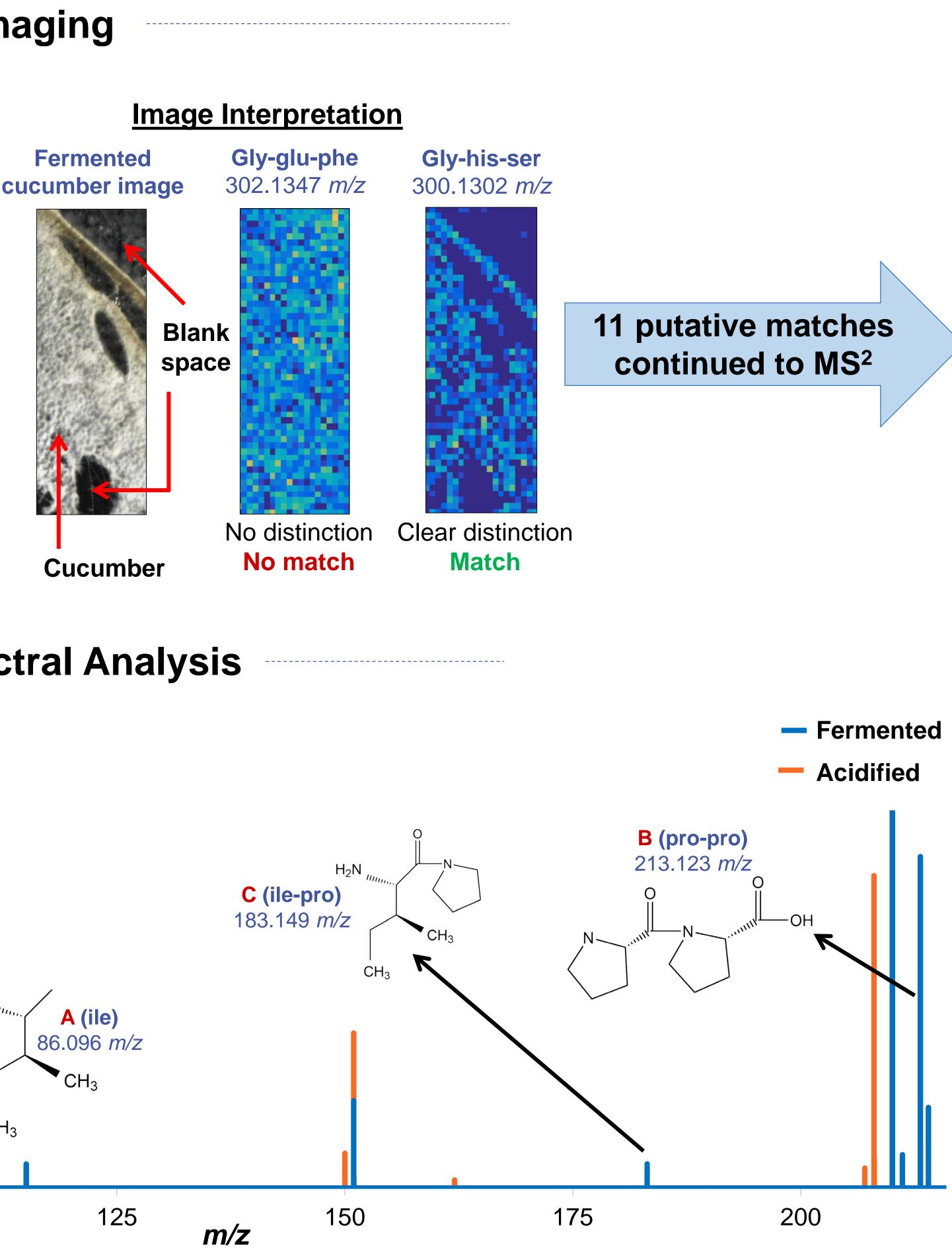
Internal database



 $\rightarrow$ Converted







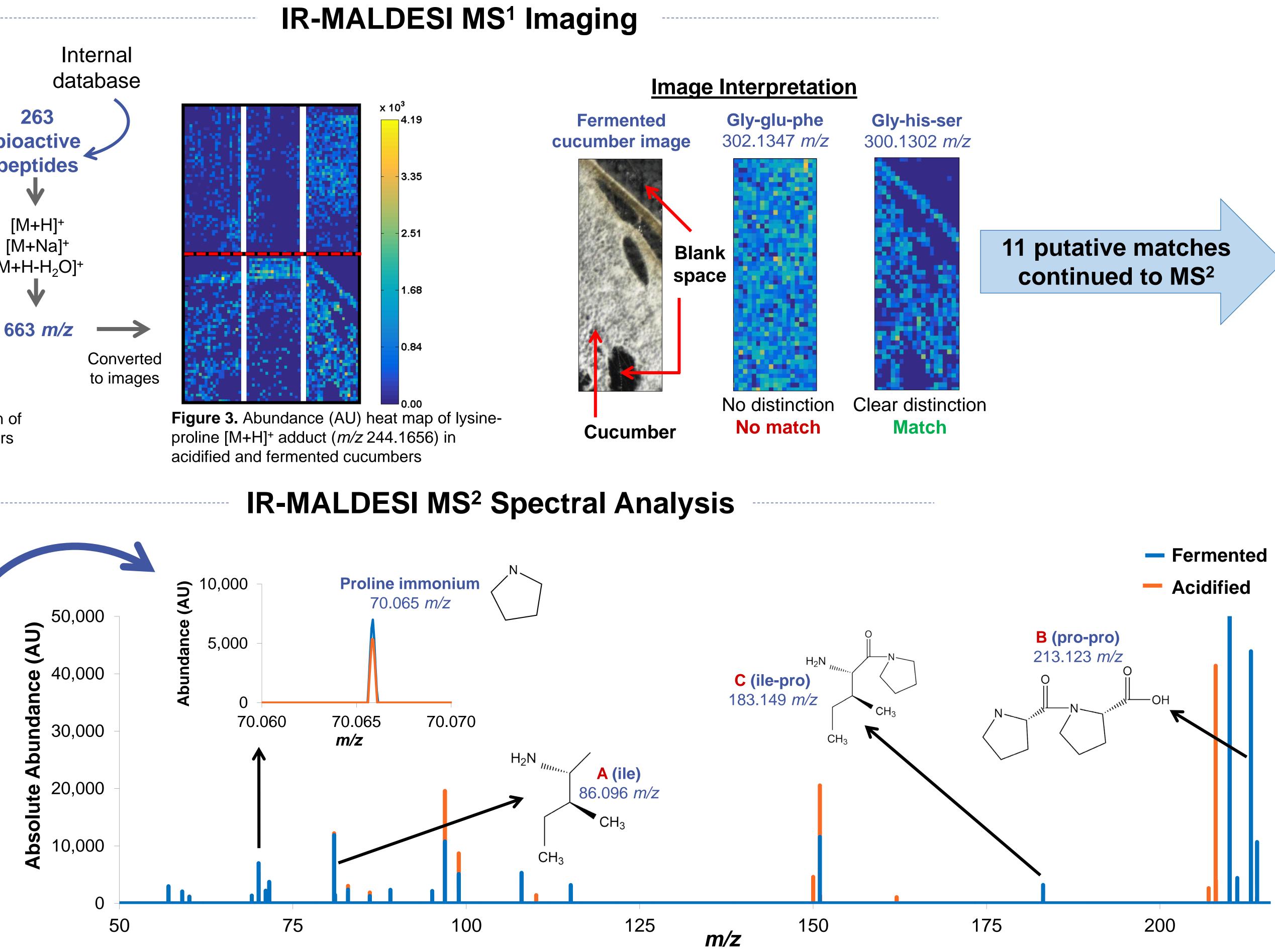


Figure 4. Average MS<sup>2</sup> spectra of the putative match for isoleucine-proline-proline<sup>‡</sup> at 326.2*m/z* ± 1Da with 140,000 nominal resolving power

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

				-				
Ŏ		Peptide	Fragments identified ( <i>m/z</i> (abundance))					
H <sub>2</sub> N <sub>11111111111111111111111111111111111</sub>				Fermented			Acidified	
CH <sub>3</sub>	Isoleucine-proline-proline 326.2074 <i>m/z</i>	<b>lle/Leu-pro-pro</b> ‡ (19.5 eV)	<b>70.065</b> (6500) <b>183.149</b> (2930)	<b>86.096</b> (2420) <b>213.123</b> (43200)	<b>116.071</b> (5930)	<b>70.065</b> (5450) <b>183.149</b> (1220)	<b>86.096</b> (1870) <b>213.123</b> (6040)	<b>116.071</b> (465)
H <sub>2</sub> N <sub>//////</sub>		Lys-pro (24.4 eV)	<b>70.065</b> (25000) <b>116.071</b> (5080)	<b>84.081</b> (66100) <b>129.102</b> (1300)	<b>86.096</b> (1410)	<b>70.065</b> (18700) <b>116.071</b> (334)	<b>84.081</b> (23600) <b>129.102</b> (1730)	<b>86.096</b> (6460)
Сн	∫ <b>Leucine-proline-proline</b> 326.2074 <i>m/z</i>	Arg-tyr (27.1 eV)	<b>70.065</b> (4760)	<b>136.076</b> (1060)	<b>175.119</b> (3390)	<b>70.065</b> (4340)	<b>136.076</b> (932)	<b>175.119</b> (247)
H <sub>2</sub> N <sub>11111111111111111111111111111111111</sub>		Val-pro-pro (18.7 eV)	<b>70.065</b> (9740) <b>213.123</b> (13600)	<b>116.071</b> (1120)	<b>169.133</b> (6150)	<b>70.065</b> (276)	<b>169.133</b> (146)	<b>213.123</b> (147)
H <sub>3C</sub>	CH₃ Valine-proline-proline 312.1918 m/z	<sup>‡</sup> Isoleucine-proline-proline an	d leucine-proline-proline h	ave the same <i>m/z</i> value	and fragmentation patte	erns. Either or both of the	se peptides may be pre	sent in the samples.

**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

# NC STATE

• Bioactive peptides were documented for the first time in a fermented vegetable