

VALIDATING THE ABILITY OF REIMS TO DIFFERENTIATE LAMB FLAVOR PERFORMANCE BASED ON CONSUMER PREFERENCE

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Rapid Evaporative Ionization Mass Spectrometry (REIMS)



REIMS

- Emerging ambient ionization technique
- Direct molecular profiling:
 - Real-time chemical fingerprint
 - No sample preparation
 - Little training



(Balog et al., 2010, 2016; Stead, 2015).

REIMS (cont.)

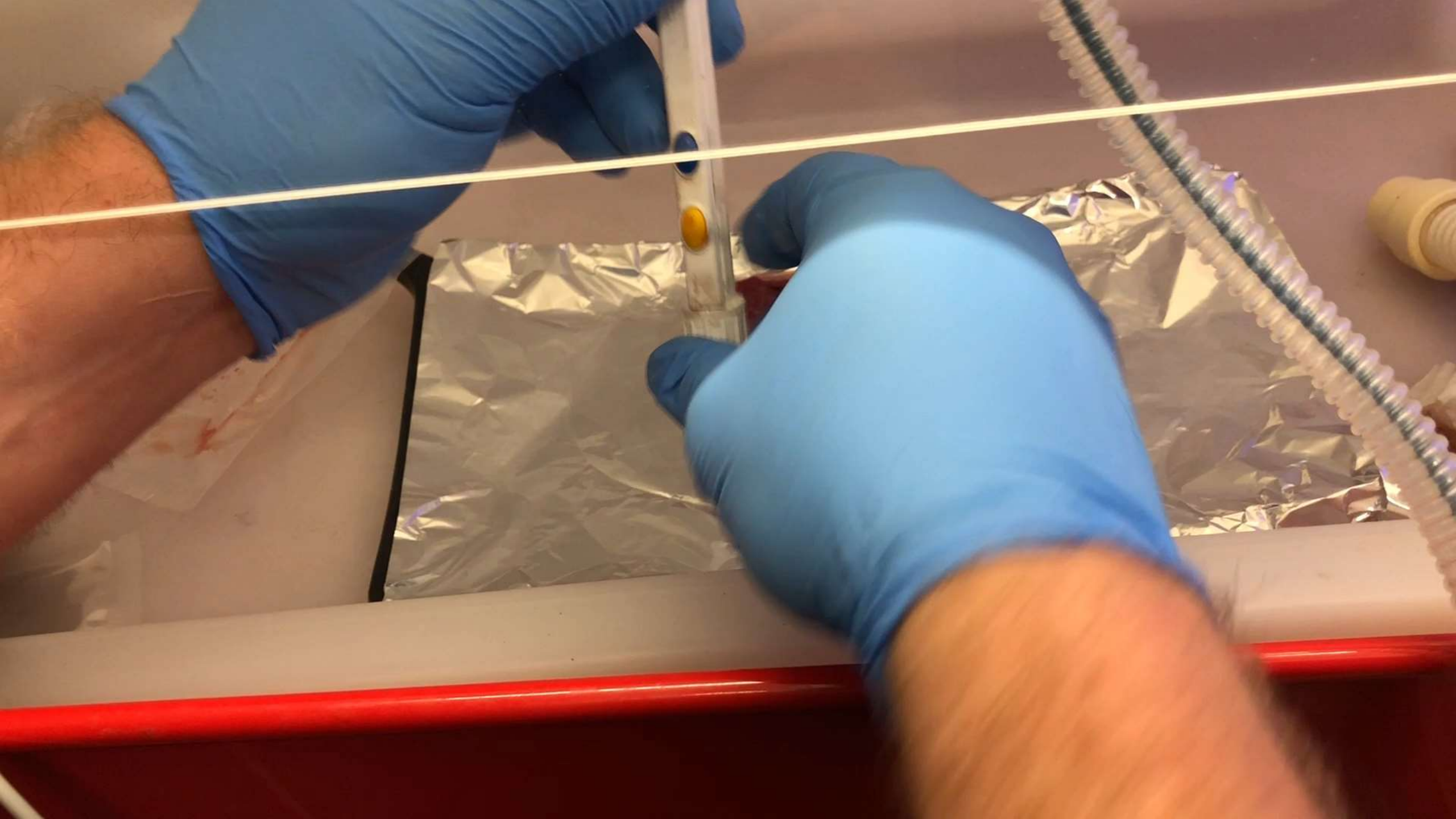
- Originally developed for the medical industry
 - Biopsies
 - Detect cancerous from non-cancerous tissues
- Adopted by the food industry
 - Food fraud
 - Specie, and tissue differentiation
 - Little in flavor



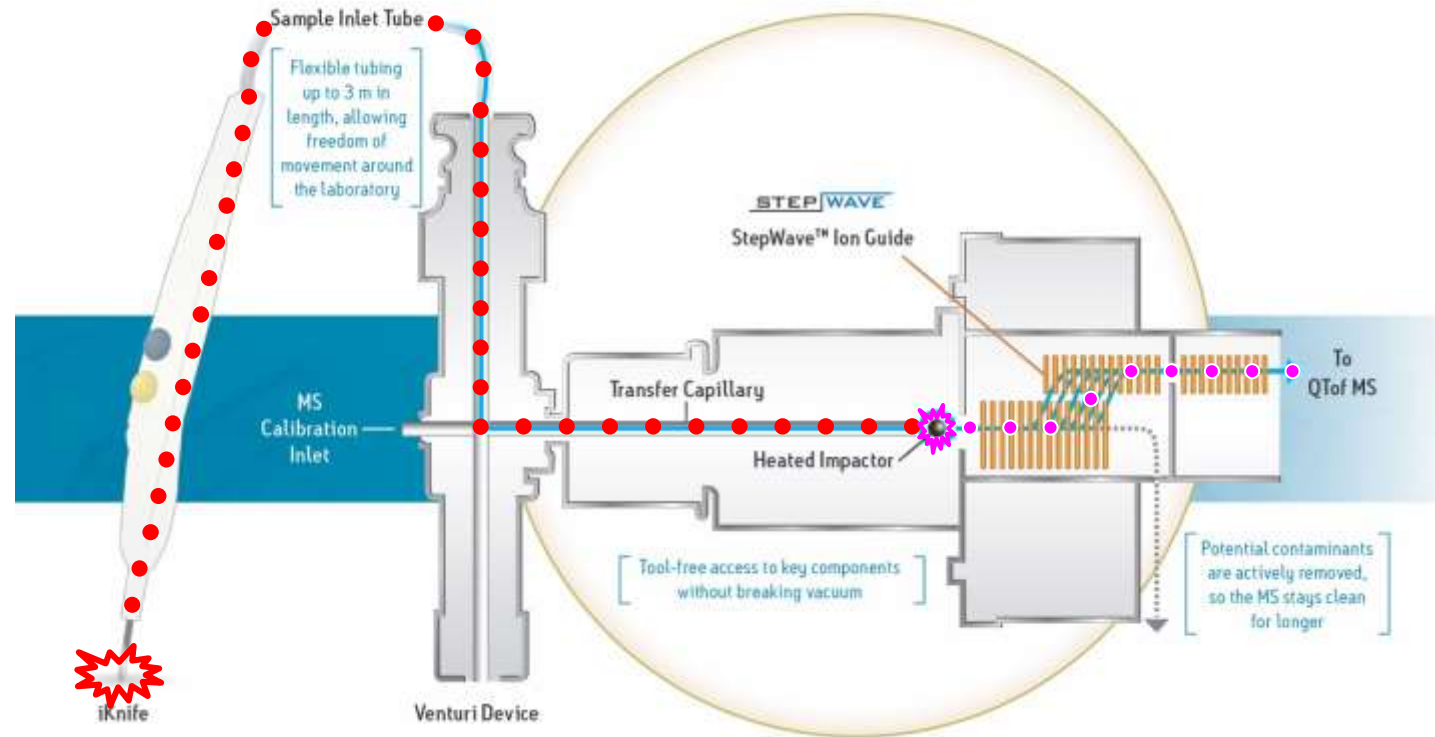
(Balog et al., 2010, 2016).







RAPID EVAPORATIVE IONIZATION (REIMS) MASS SPECTROMETRY

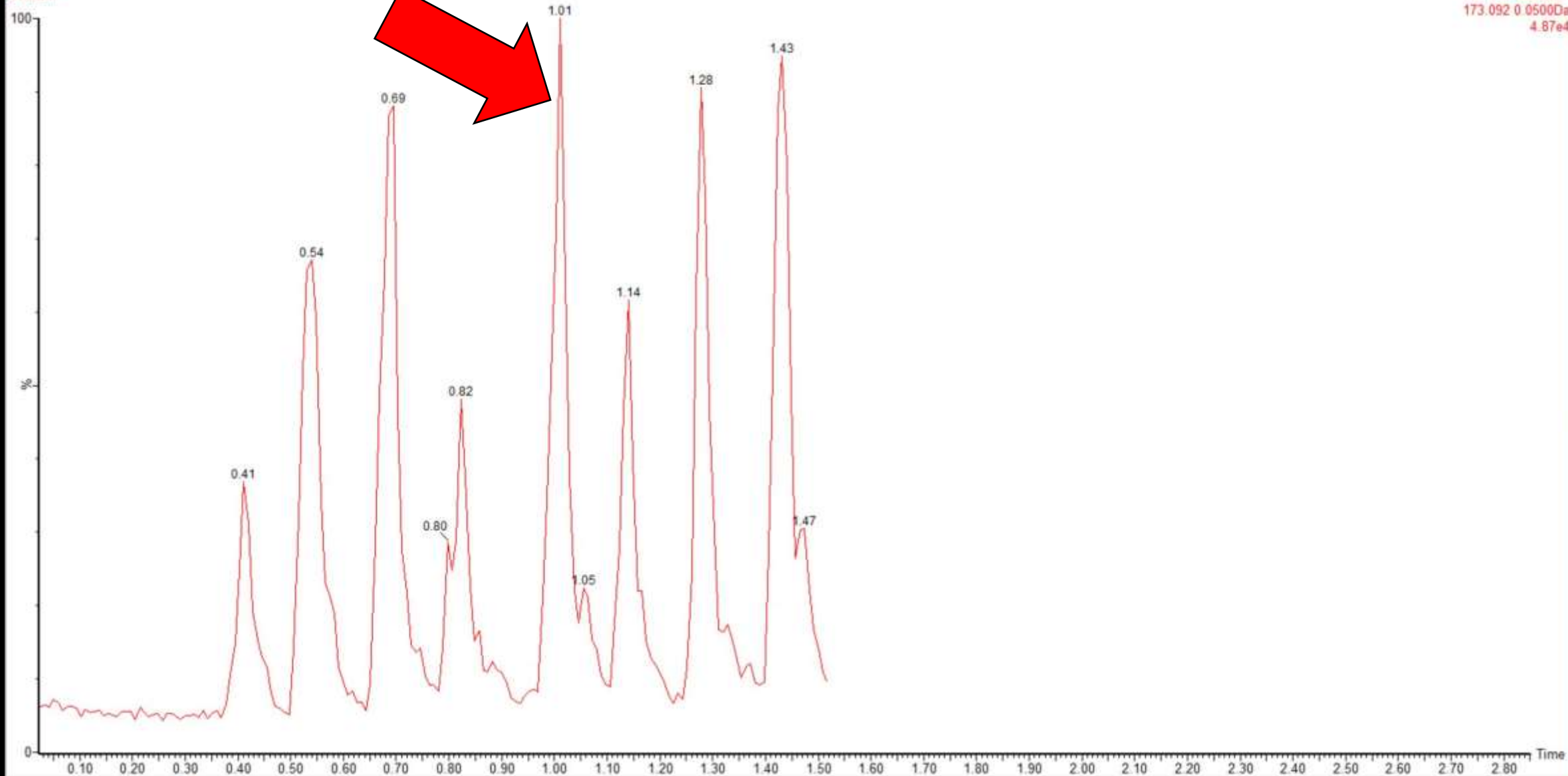


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SYNAPT G2-Si#UGB732

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TOF MS ES-
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4.87e4



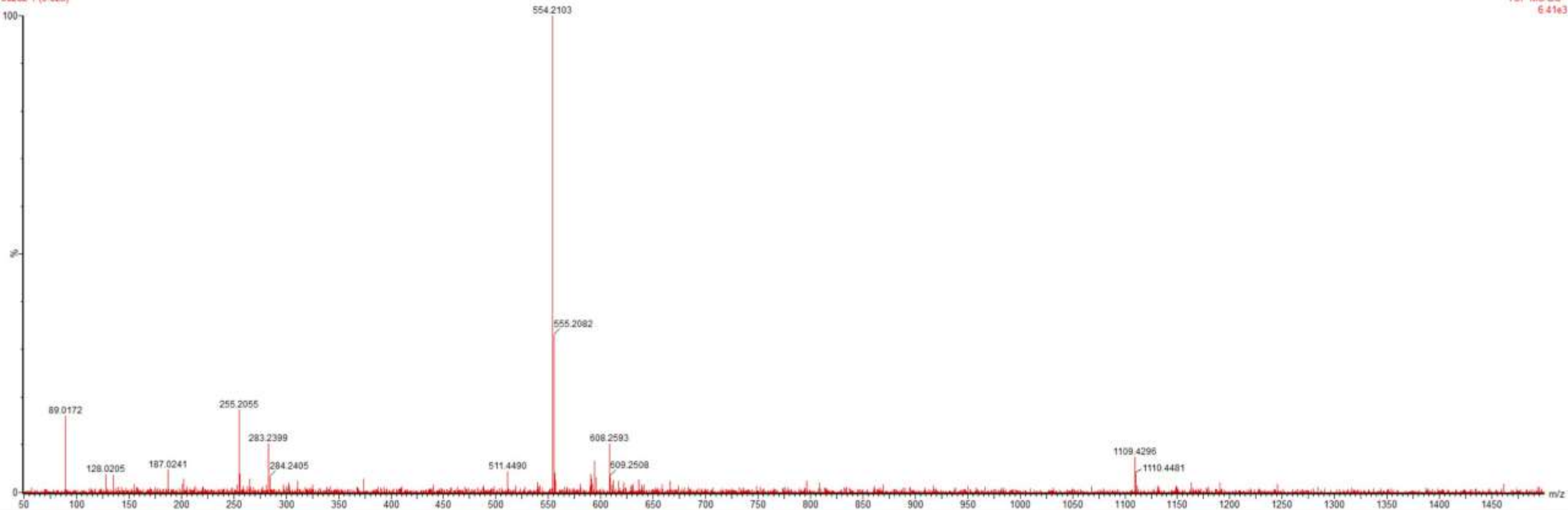
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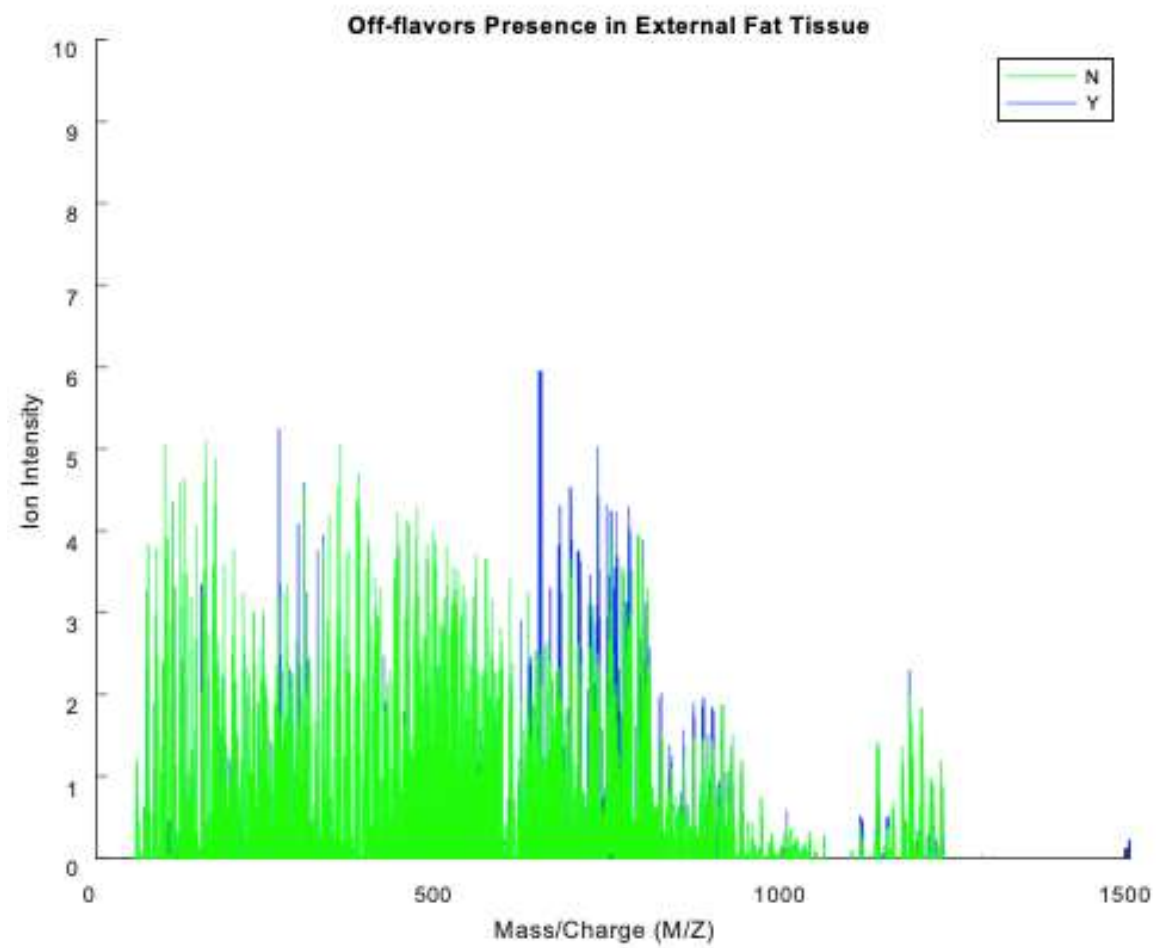
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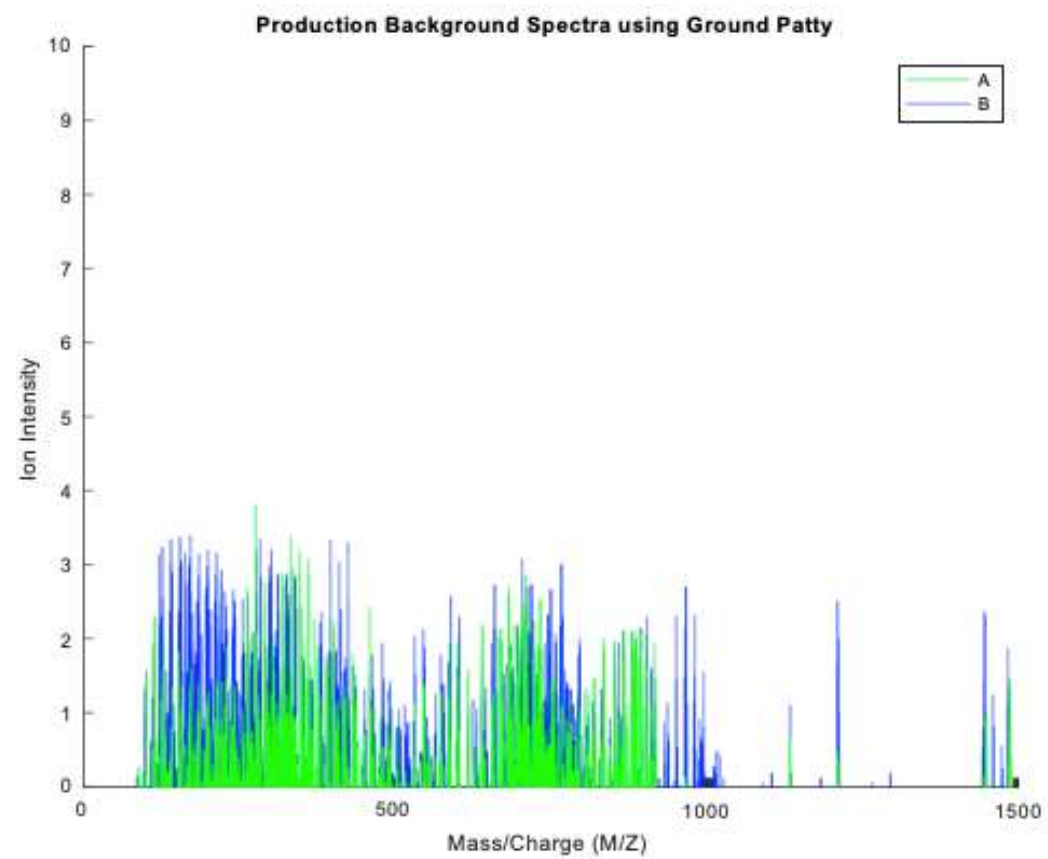
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TOF MS ES-

6.41e3







Materials and Methods



Production Facility

- Dixon, CA
- Denver, CO
- Greeley, CO

Variety of:

- Production seasons
- Production backgrounds
- Sex classifications
- Breeds

Age

- Lamb (n=99)
- Yearling (n=101)

Sample Collection
(N=200)



Guide to estimating the age of sheep by their teeth

| | | |
|---|---|--|
|  | Birth to 12 months Lamb's teeth | 8 milk teeth |
|  | 12-19 months Two-tooth | 2 central incisors 6 milk teeth |
|  | 18-24 months Four-tooth | 2 central incisors 2 middle incisors 4 milk teeth |
|  | 23-36 months Six-tooth | 2 central incisors 2 middle incisors 2 lateral incisors 2 milk teeth |
|  | 28-48 months Eight-tooth | 2 central incisors 2 middle incisors 2 lateral incisors 2 corner incisors |

Sample Collection

REIMS Sample

- 10X10 cm area sample from biceps femoris
- Placed into whirl pack bag
- Shipped on dry ice to Texas Tech Meat lab
- Stored in -80° C

Remaining leg sample

- left in the cooler for fabrication
- Boneless leg samples (Institutional Meat Purchase Specifications #234,2014)

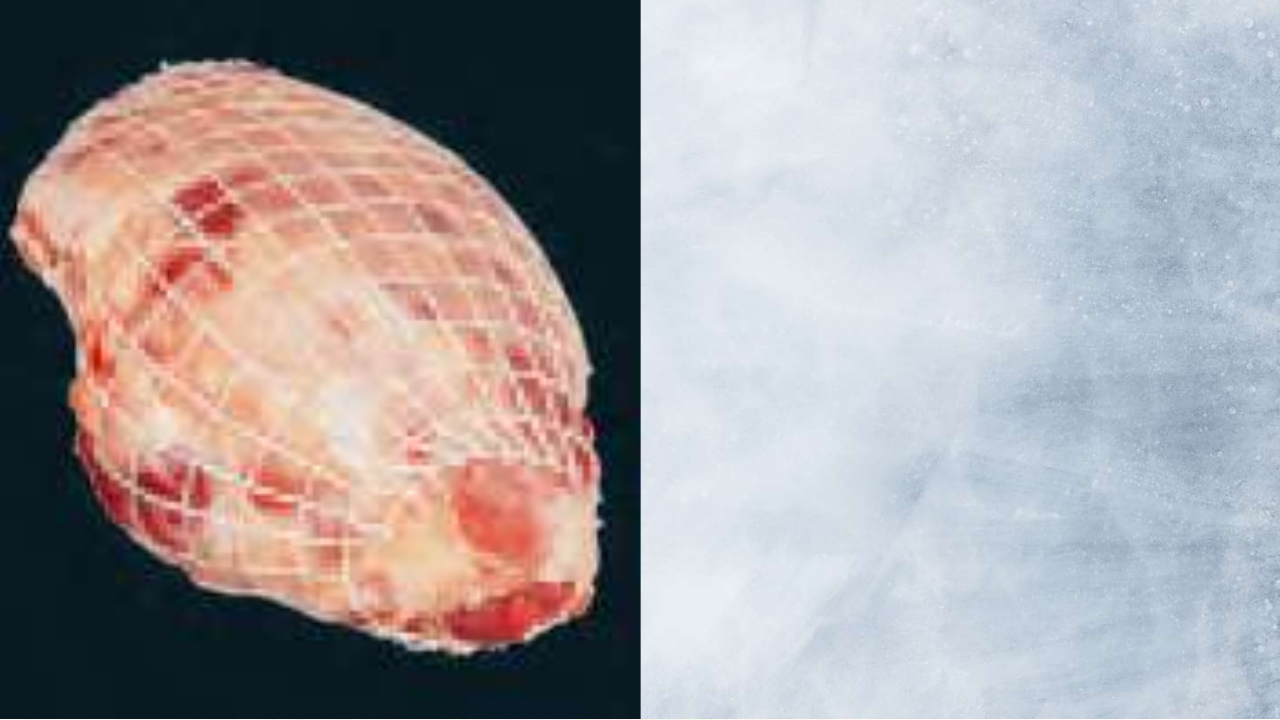


Leg Fabrication

- 7-day postmortem aging

Specifications

- All external fat was trimmed (<0.0 cm)
- Seam fat were removed
- Present lymph glands were removed



Patty Formation



- Each leg was ground using:
 - 12mm coarse grind plate
 - 3mm fine grind plate
- Using the Eazy Slider Patty-O-Matic
 - one ounce
 - $\frac{3}{4}$ inches thick

Consumer Sensory Panels

- Total panelists n=200
- 18-70 years old

Panels

- 10 days
- 2 panels per night
- Each panel consisted of 20 consumers
- 45 minutes
- Each panelist was provided the following:
 - Palate Cleansers: unsalted crackers, Water, Diluted apple juice (90:10 dilution)
 - Utensils: fork, knife, toothpick



Rapid Evaporative Ionization Mass Spectrometry (REIMS)

- Prior to analysis
 - For 6 to 8 hours
 - At 0 to 4 °C



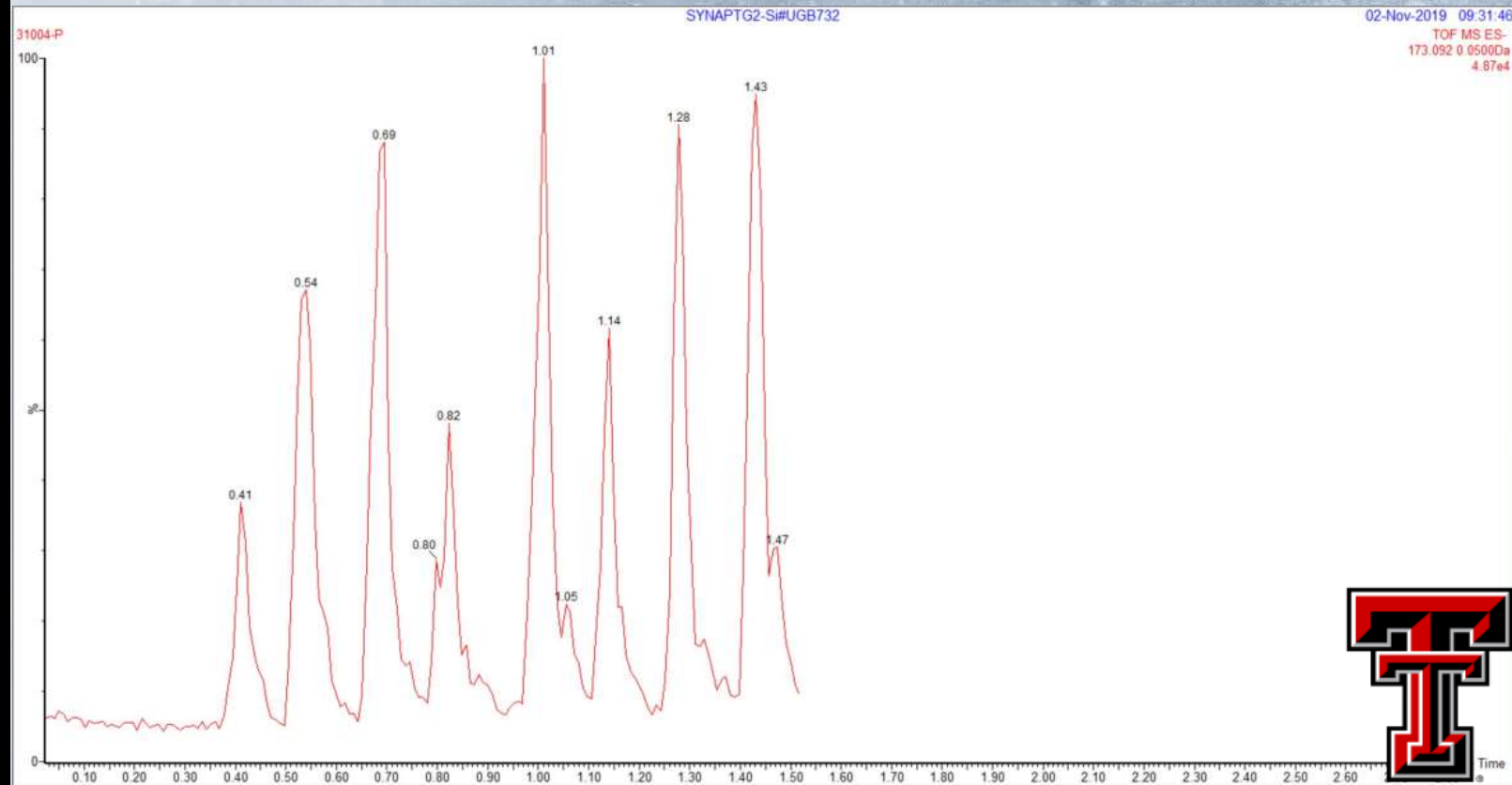
- At least five burns made lasting 1-2 seconds
 - Lean tissue
 - Fat tissue
 - Sensory patties



Abstract Model Building (AMX) software



- Data preprocessing
 - lock mass (leucine enkephalin at 554.25 m/z) correction
 - Background subtraction
 - Normalization of total ion current



Statistical Analysis



Sheep Characteristics

- One-way analysis of variance (ANOVA)
- Determine the impact of a sole response
- Significant differences

Production background

Sex characteristics

Age Classifications

&

Flavor intensity

Overall flavor

Overall liking



Predictive Model Descriptions

Models included

- Off-flavors
- Overall liking
- Flavor intensity
- Flavor overall
- Production background (grain-fed and grass-fed)
- Sex classifications (ewe; wether; ram)
- Age classifications (Lamb and Yearling)



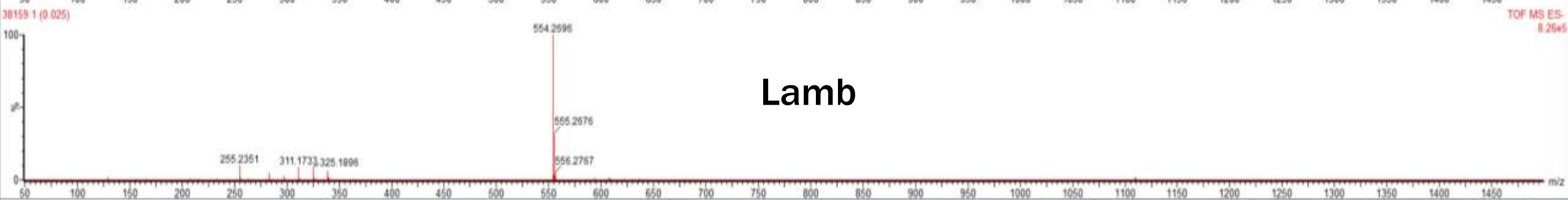
Results and Discussion



Sheep Characteristics

| Treatment Group | n | Flavor Intensity | Overall Flavor | Overall Liking |
|-----------------------|-----|------------------|-----------------|-----------------|
| Production Background | | P=0.6463 | P=0.0710 | P=0.1552 |
| Grain- fed | 99 | 49.93 | 49.17 | 50.90 |
| Grass- fed | 101 | 49.23 | 51.85 | 53.11 |
| SEM | | 1.08 | 1.05 | 1.10 |
| Sex Classification | | P=0.3999 | P=0.4706 | P=0.1110 |
| Ewe | 96 | 48.52 | 49.50 | 50.74 |
| Wether | 85 | 50.41 | 51.59 | 50.05 |
| Ram | 19 | 51.18 | 49.68 | 53.89 |
| SEM | | 2.49 | 2.67 | 2.50 |
| Age Classification | | P=0.6652 | P=0.2764 | P=0.0810 |
| Lamb | 99 | 49.91 | 49.70 | 50.65 |
| Yearling | 101 | 49.25 | 51.33 | 53.36 |
| SEM | | 1.11 | 1.06 | 1.13 |





Consumer Demographics and Questionnaire

Gender

- 59.0% Female

Ethnicity

- 65.0% Caucasian/White
- 30.0% Hispanic

Age

- 65.0% 20-49 years old

Marital Status

- 61.0% single

Education

- 79.0% had at least some college/technical school

Salary

- 75.5% at most \$50,000-\$74,999

Consumption of red meat

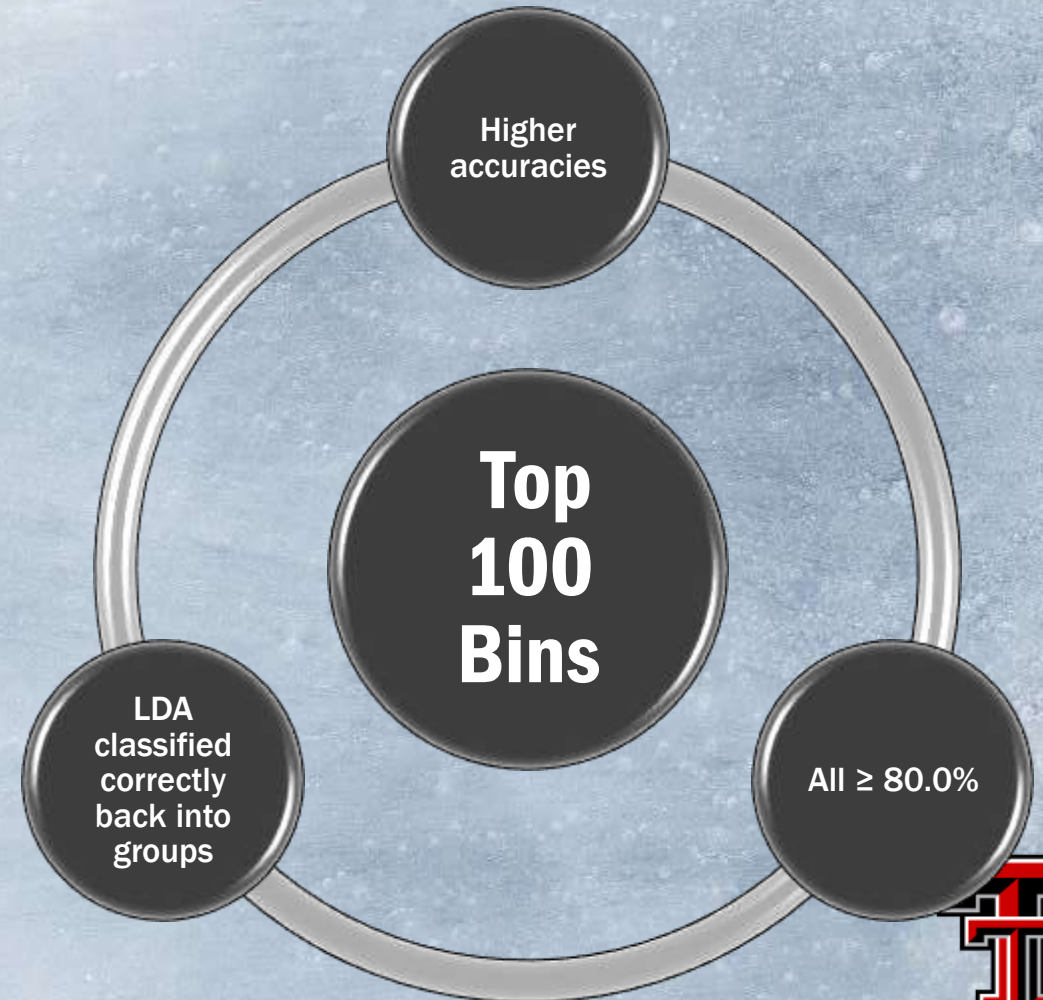
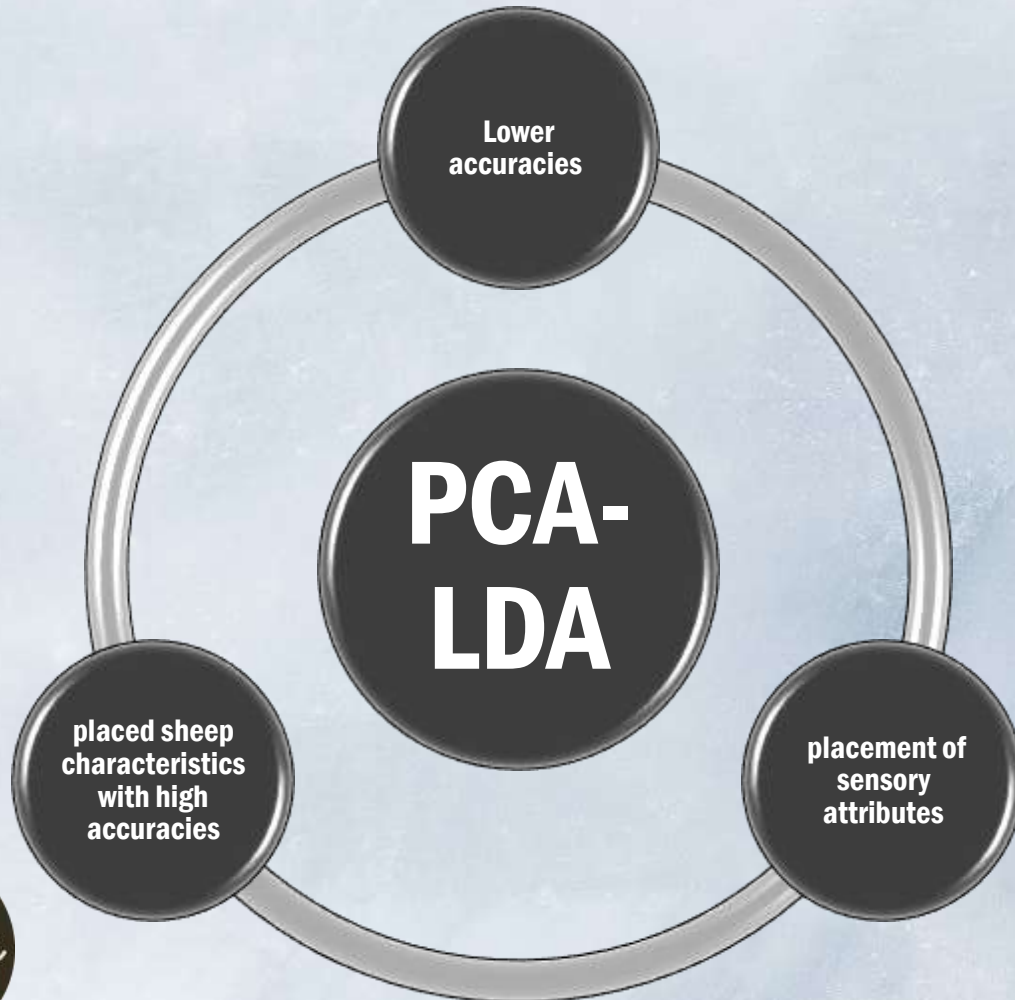
- 95.0% 1-14 times per week
- majority ate red meat 1-6 times per week (66.5%)

Consumption of sheep meat

- 57.5% Never/rarely
- 71.5% consumed sheep once a year or less



Comparison of Predictive Models



Top 100 REIMS Bins

Lean tissue,
external fat tissue,
ground patty

Sheep
Characteristics

Sensory
Attributes

- All $\geq 80\%$ overall accuracy

Off-flavors

- Lean tissue 86.66%
- External fat tissue 92.64%
- Ground patty 86.50%

Production background

- Highest accuracies for all sample types
 - Lean tissue and ground patties = 100.00%
 - External fat tissue = 99.50%



Top 100 REIMS Bins

Lean tissue,
external fat tissue,
ground patty

Sheep
Characteristics

Sensory
Attributes

- Lean accuracies were highest classifying:
 - flavor intensity acceptability (89.0%)
 - flavor intensity classes (89.0%)
 - age classification (88.0%)
- Patty accuracies were highest classifying:
 - overall liking acceptability (86.00%)
 - overall flavor acceptability (89.00%)
 - sex classifications (85.00%)



**Correlations
between top
REIMS, sheep
characteristics,
flavor
attributes,
metabolites,
and volatile
compounds**

Searched compounds:

1. Lean tissue
2. External fat tissue
3. Ground sensory patty

All identified compounds correspond to previously reported compounds found in lamb and meat products



Production Background

| Lean tissue | External fat tissue | Ground sensory patty |
|--|---|---|
| Positively correlated (+) | | |
| Grain-fed | | |
| <ul style="list-style-type: none"> • BCFA <ul style="list-style-type: none"> ➤ Mild flavor • Indoles | <ul style="list-style-type: none"> • BCFA <ul style="list-style-type: none"> ➤ Mild flavor | <ul style="list-style-type: none"> • BCFA <ul style="list-style-type: none"> ➤ Mild flavor • Indoles • Gamma-dodecalactone <ul style="list-style-type: none"> ➤ Corn fed • Propionate |
| Grass-fed/pasture raised | | |
| <ul style="list-style-type: none"> • eicosapentanoic acid (EPA) <ul style="list-style-type: none"> ➤ PUFA (Leukotriene) • Terpenes <ul style="list-style-type: none"> ➤ In plants • Alpha-linolenic • palmitoleic acid | <ul style="list-style-type: none"> • eicosapentanoic acid (EPA) <ul style="list-style-type: none"> ➤ PUFA (Leukotriene) • linolenic acid • Terpenes <ul style="list-style-type: none"> ➤ In plants | <ul style="list-style-type: none"> • linolenic acid (alpha-linolenic acid) <ul style="list-style-type: none"> ➤ Flavor intensity ➤ Pastoral flavor • Delta-dodecalactone • Oleic acid <p>Green/grassy notes</p> <ul style="list-style-type: none"> • 4-heptenal and 2-heptadecanone |
| Negatively correlated (-) | | |
| <ul style="list-style-type: none"> • Oleic acid • Aspartic acid | <ul style="list-style-type: none"> • Gamma/delta-dodecalactone • 4-methyloctanoic acid (mutton odor) • Stearic acid (lamb ≥ 12 months) • Aspartic acid | <ul style="list-style-type: none"> • eicosapentanoic acid (EPA) |

Age Classification

| Lean tissue | External fat tissue | Ground sensory patty |
|---|---|---|
| Age classes | | |
| Positively correlated (+) | | |
| Indoles <ul style="list-style-type: none"> ➤ increase with maturity ➤ negative impact on flavor Gamey flavors <ul style="list-style-type: none"> • Methionine, aspartic acid Cysteine Arachidonic acid | Indoles <ul style="list-style-type: none"> ➤ increase with maturity ➤ negative impact on flavor Pentadecanoic acid <ul style="list-style-type: none"> ➤ directly correlated to age 2-pentylpyridine <ul style="list-style-type: none"> ➤ Mutton odor Gamey flavors <ul style="list-style-type: none"> • Methionine, aspartic acid Leukotriene 1-pentanol <ul style="list-style-type: none"> ➤ Mild odor | BCFA Indoles <ul style="list-style-type: none"> ➤ increase with maturity ➤ negative impact on flavor Gamey flavors <ul style="list-style-type: none"> • Methionine, aspartic acid Cysteine |
| Negatively correlated (-) | | |
| Thymol <ul style="list-style-type: none"> ➤ Common in sheep meat Heptadecanoic acid 1-octen-3-one | Toluene Cysteine Oleic acid | 2-methylbutanal <ul style="list-style-type: none"> ➤ Important in grilled lamb |

Sex Classification

| Lean tissue | External fat tissue | Ground sensory patty |
|--|---|--|
| Sex classes | | |
| Positively correlated (+) | | |
| BCFA Indoles <ul style="list-style-type: none"> ➤ Mutton, sheepy Linolenic acid <ul style="list-style-type: none"> ➤ Found in rams Heptadecanoic acid <ul style="list-style-type: none"> ➤ Identifier of age Toluene | BCFA Indoles <ul style="list-style-type: none"> ➤ Mutton, sheepy Glyceric acid <ul style="list-style-type: none"> ➤ Gamey 2-mercapto-3-pentanone <ul style="list-style-type: none"> ➤ meaty | BCFA Indoles <ul style="list-style-type: none"> ➤ Mutton, sheepy Gluconic acid Glucuronic acid Bornyl acetate Cysteine Phenylalanine |
| Negatively correlated (-) | | |
| Prostagldins, leukotrienes, stearic acid and pentadecanoic acid <ul style="list-style-type: none"> ➤ Rams fat Terpenes | Toluene | Ethyl hexanoate (butter, meaty, cheese-like, fried-oil like) 2-butyl-2-octenal (meaty) Vanillin (vanilla) |

Flavor

| Lean tissue | External fat tissue | Ground sensory patty |
|---|---|--|
| Flavor | | |
| Positively correlated (+) | | |
| Sulphur containing compounds 4-methyloctanoic acid (MOA) (mutton) Indoles Grass-fed <ul style="list-style-type: none"> ➤ Pentanoic acid ➤ Heptanoic acid ➤ Gluconic acid (universal indicator of grass flavor) ➤ 2-hexenal ➤ Terpenes Hypotaurine (bitter) PUFA (higher in rams) Arabitol | Sulphur containing compounds <ul style="list-style-type: none"> ➤ 4,5-dimethylthiazole (pungent) ➤ Dimethyl trisulfide (roast, musty) 3,5-dimethyl-1,2,4-trithiolane (mutton aroma) | Lactones <ul style="list-style-type: none"> ➤ Roast Negative attributes <ul style="list-style-type: none"> ➤ Cysteine (metallic, sulfurous) ➤ Methionine (gamey) ➤ Indoles ➤ Eicosapentaenoic acid (EPA) ➤ Toluene ➤ (E,E) 2,4-dodecadialenal |
| Negatively correlated (-) | | |
| (E,E) 2,4-dodecadialenal Linolenic acid Ethyl hexanoate (butter) 4-heptenal 2-methylbutanal (roast) 2,4-dodecadialenal (roast) 2-deceanal (roast, fatty) | (E,E) 2,4-dodecadialenal 4-heptanone 2-methyl-3-furanthiol (E,E) 2,4-decadialenal (meaty) Prostaglandin Vanillin | Sulphur containing compounds Glycine (sweet note) |

Flavor Intensity

| Lean tissue | External fat tissue | Ground sensory patty |
|--|---|--|
| Flavor Intensity | | |
| Positively correlated (+) | | |
| ANOVA groups Mutton odors Arabitol Terpenes Meaty flavors <ul style="list-style-type: none"> ➤ (E,E)- 2,4-decadienal ➤ 2-heptenal 2-pentylfuran (spicy) | ANOVA groups Meaty flavors (-thiols) 3,5-dimethyl-1,2,4-trithiolane (mutton odors) 2-isopropylphenol (sheep meat flavor/odor) | ANOVA groups Stearic acid 2,4-dodecadienal Decanoic |
| Levels of intensity Hypotaurine (bitter) Toluene PUFA Gluconic (gamey) | Levels of intensity Gamey <ul style="list-style-type: none"> ➤ Methionine ➤ Cysteine ➤ Glucuronic | Levels of intensity Hexadecenoic acid Lactones Phenols |
| Negatively correlated (-) | | |
| ANOVA groups Roasty, meaty, mild flavor compounds Pastoral, gamey, mutton, strong flavor and odor <ul style="list-style-type: none"> ➤ 4-methyloctanoic acid ➤ 4-methyl phenol (p-Cresol) | ANOVA groups Roasty, meaty flavors <ul style="list-style-type: none"> ➤ 2-methylbutanal ➤ 2-butyl-2-octenal | ANOVA groups BCFA Oleic acid Vanillin |
| Levels of intensity Pentadecanoic acid Decanoic acid | Levels of intensity Linolenic Thymol | Levels of intensity Stearic acid |

Off-Flavors

| Lean tissue | External fat tissue | Ground sensory patty |
|--|---|--|
| Off-flavors | | |
| Positively correlated (+) | | |
| Terpenes Pungent lamb odor <ul style="list-style-type: none"> ➤ 2,4-dimethylthiazole ➤ 4,5-dimethylthiazole Known to reside in sheep meat <ul style="list-style-type: none"> ➤ 2-methylbenzaldehyde ➤ 2-ethylphenol ➤ 2,4-dimethylphenol | Pungent lamb odor <ul style="list-style-type: none"> ➤ 2,4-dimethylthiazole ➤ 4,5-dimethylthiazole Hypotaurine (bitter) Heptanoic acid (grass-fed) | Lactones Indoles Eicosapentaenoic acid (EPA) Heptadecanoic acid Cysteine Malonate |
| Negatively correlated (-) | | |
| Hypotaurine Cysteine | Mutton flavor/odor <ul style="list-style-type: none"> ➤ 4-methylphenol (p-cresol) ➤ 3,5-dimethyl-1,2,4-trithiolane Arachidonic acid | Stearic Gamma/delta dodecalactone |

Overall Liking

| Lean tissue | External fat tissue | Ground sensory patty |
|---|---|--|
| Overall liking | | |
| Positively correlated (+) | | |
| -thiols -thiazoles Meaty, fatty, roasty characteristics <ul style="list-style-type: none"> ➤ 2,4-decadienal ➤ Dimethyl trisulfide ➤ (E) 2-butyl-2-octenal, furaneol (meaty) Linolenic acid | Delta/gamma-dodecalactone Roast, meaty <ul style="list-style-type: none"> ➤ 2-methylbutanal ➤ (E)-2-butyl-2-octenal ➤ 2-decenal ➤ 2-ethyl-3,5-dimethylpyrazine Pentanal Terpenes 2-hexenal | Thiazoles Pyrazines Lactones (roast) |
| Negatively correlated (-) | | |
| 3-methylindole (animal, rancid) 4-heptenal (pasture-fed) Toluene (pasture-fed) Heptanoic acid (grass-fed) Delta/gamma-dodecalactone Eicosapentaenoic acid Pyruvic acid (gamey) | 4-methyloctanoic acid (MOA) 4-heptanone (grain-fed) Arabitol (metallic) 1-octen-3-one (mushroom) Meaty characteristics | (E,E)2,4-dodecadienal, Hexanoic acid Delta/gamma-dodecalactone |

Conclusion



- **REIMS can accurately predict and classify multiple factors**
 - Sheep characteristics
 - Sensory attributes
 - Top 100 REIMS bins model revealed higher accuracies than PCA-LDA model
- **Possible compounds found from the database**
 - Discovered known compounds affecting flavor, odor, and biomarkers for certain characteristics
 - 4-methyloctanoic acid (MOA), 3-methylindole→ decrease overall liking, negative eating experience



What's next?

In-plant use:

- Differentiate lamb flavor in real-time
- Be categorized by flavor intensity
- Accurately evaluate value of lamb carcasses
- Institute branded programs
- Widen the market
- Increase demand



Questions?



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