Use of machine learning and REIMS technology to improve beef quality

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"Old" approach—Yield grade equation (Murphey et al., 1960)

% BCTRC = $51.34 - (5.784 \times AFT) - (0.462 \times KPH) + (0.74 \times REA) - (0.0093 \times HCW)$ YG = $2.50 - (2.50 \times AFT) - (0.20 \times KPH) + (0.32 \times REA) - (0.0038 \times HCW)$

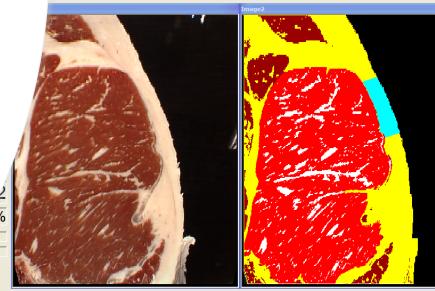
- Linear regression
- Small number of variables
- Known relationships to trait of interest

"New approach"—yield grading

- Collect image of ribeye at 12th-13th rib
- ~20,000 variables
- ???Relationship to yield
- Find previously unknown relationships
- Al to better segment the ribeye



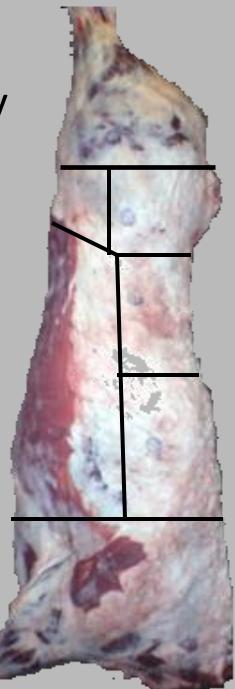
and Settings\shackelford\My Documents\E+V\images\00183755.tif



ID	183755
Wgt	815.0
PYG	3.2
ADJ	3.2
REA	16.8
YG	1.9
Marb	Md 60

Machine learning in meat industry

- Process optimization
- Automation
- Value determination
- Research and Development— Understanding biology



Understanding regulation of the phenotype

- Genetics
- Nutrition
- Time on feed
- Growth promotants
- Metabolic modifiers
- Animal age

- Electrical stimulation
- Chilling rate
- State of contraction
- Postmortem aging
- Blade tenderization
- Marination

We have studied these factors and more to understand their effects on tenderness, color stability, healthfulness, ...

Component traits

Majority of variation left unexplained

WHAT ARE WE MISSING?

Table 3. Pearson correlation coefficients between ten-derness traits in gluteus medius steaks

Item	Slice shear force	$\begin{array}{c} { m Sarcomere} \\ { m length} \end{array}$	Desmin degraded
All aging times			
combined			
Overall tenderness	-0.63^{***}	0.23^{*}	0.36^{***}
Slice shear force		-0.47^{***}	-0.47^{***}
Sarcomere length			0.07
14 d postmortem			
Overall tenderness	-0.61^{***}	0.43^{**}	0.12
Slice shear force		-0.59^{***}	-0.18
Sarcomere length			-0.01
42 d postmortem			
Overall tenderness	-0.49^{***}	0.10	0.19
Slice shear force		-0.48^{***}	-0.05
Sarcomere length			0.05

*P < 0.05; **P < 0.01; ***P < 0.001.

Table 8

Partial regression correlation coefficients for the relationship between oxygen consumption and metmyoglobin reducing activity measurements and changes in color parameters of beef longissimus steaks when measured at the initiation or conclusion of 6 d of simulated retail display.

Variable	Oxygen consumption		Nitric oxide metmyoglobin reducing activity ^a		Initial metmyoglobin formation ^b		Post-reduction metmyoglobin ^c	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
Day 6 a*	-0.25***	-0.11	0.27***	0.35***	-0.17**	-0.15*	-0.27***	-0.27***
Day 6 K/S572/K/S525d	-0.20**	-0.12	0.38***	0.39***	-0.22***	-0.13*	-0.38***	-0.41***
Day 6 Chroma	-0.24***	-0.12	0.22***	0.29***	-0.10	-0.14*	-0.22***	-0.32***
Day 6 Hue angle	0.19**	0.02	-0.38***	-0.40***	0.41***	0.13*	0.42***	0.44***
ΔE ^e	0.19**	0.12	-0.44***	-0.50***	0.23**	0.11	0.45***	0.52***
∆a*	0.17*	0.12	-0.43***	-0.50***	0.23***	0.13*	0.43***	0.52***

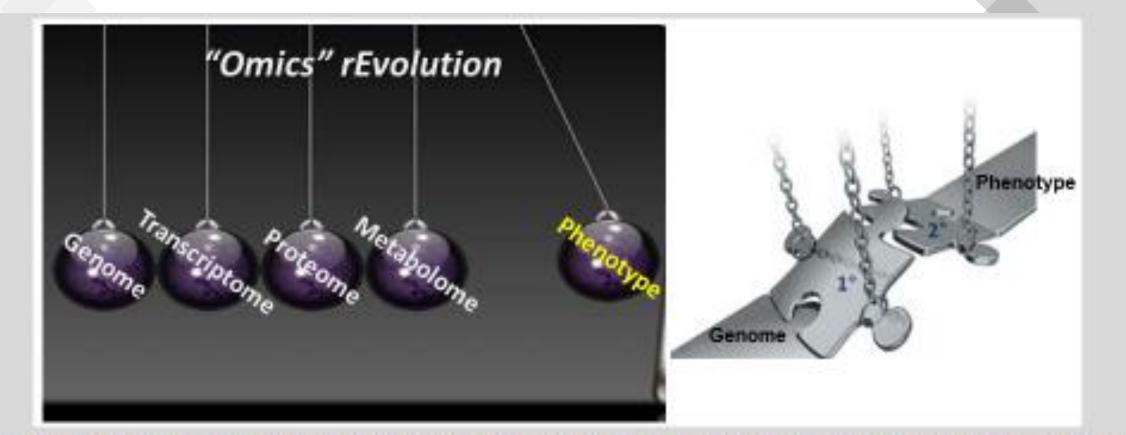
* *P*<0.05; ***P*<0.01; ****P*<0.001.

^a Nitric oxide metmyoglobin reducing activity; Initial met myoglobin formed – post-reduction metmyoglobin.

^b Initial metmyoglobin formed during 30 min incubation with 0.3% sodium nitrite.

^c Post-reduction metmyoglobin; nitric oxide met myoglobin remaining after 2 h in vacuum package.

^d Ratio of reflectance at 572 nm to 525 nm after K/S transformation, lower values indicate greater metmyoglobin content.

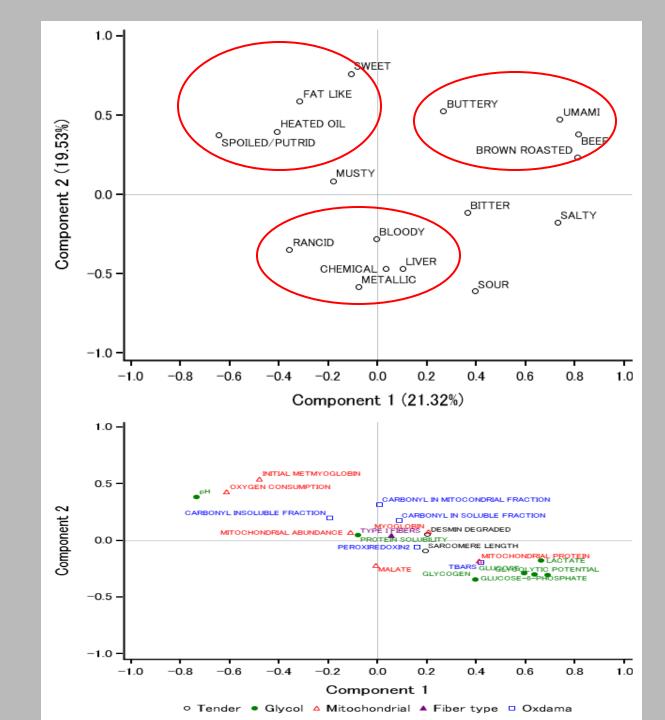


"...the best way to predict an individual's disease risk is likely to come from understanding the biology behind each disease – and that will come from a combination of genomics, proteomics, and metabolomics."

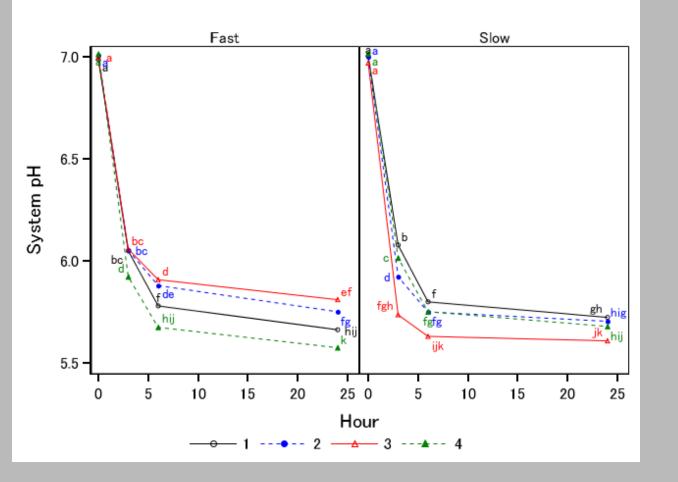
-Nature News, 2007; 446:8.

Principal Component Analysis

- One of the most common techniques for seeing complex relationships in data
- Derives a series of variables (components) that explain variation in the original data set
- First one explains the most, etc.
- By plotting loadings of variables for each component can visualize relationships



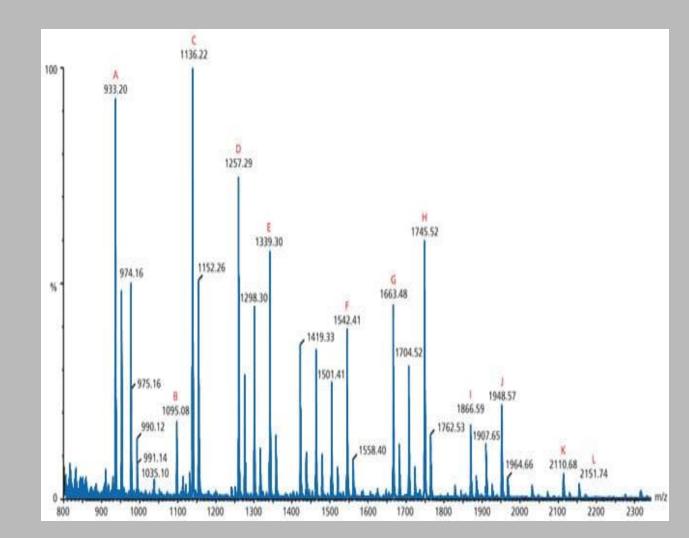
Using clustering to identify groups of samples that behave similarly in in vitro system



- Differences in enzyme system among cluster groups
- Studying differences in metabolic capacity
- Very different across temp decline rates
- Correlated to pH decline with fast temp decline
 - IMF, OC, carbonyls in mitochondrial fraction
- Correlated at slow temp decline
 - Glucose, MHCI

Mass spectrometry

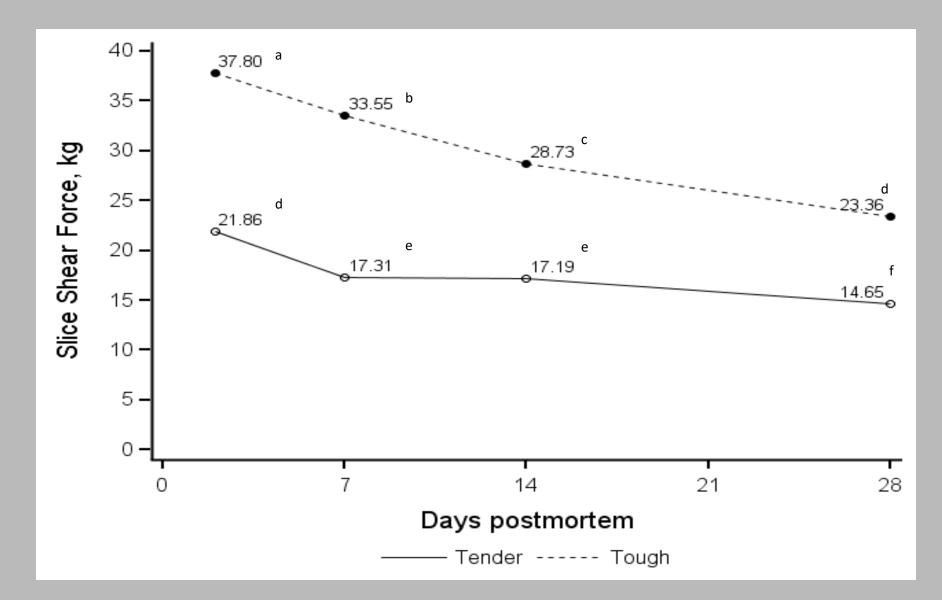
- Can be used for metabolomics or proteomics
- Requires extensive sample prep
- Time consuming
- Molecular fingerprint
- Compounds identified by libraries
- Annotation is limiting



Beef tenderness metabolomic experiment

- Carcasses selection
 - U.S. Select
 - Screened for extremes for tenderness (n = 10/class)
- Tenderness assessed using slice shear force (Shackelford et al. 1999)
- Postmortem proteolysis of desmin (Wheeler et al. 2002)
- Non-targeted proteomic profiling
 - UPLC-MS and GC-MS
- Relationships to tenderness assessed
 - ANOVA
 - Pearson correlation coefficients
 - Partial least squares regression

Slice shear force



Metabolomics results

- 2,562 metabolites profiled
- 651 met Bonferroni corrected significance level for aging effect
 - 254 met nominal significance for tenderness class effect

Significance level	Tenderness class	Aging effect	Tenderness class X aging effect
P < 2 × 10 ⁻⁵	3	651	32
P < 0.05	254	651	173

- Highly significant metabolites related to days postmortem
- Evaluated correlations with and without days postmortem effect

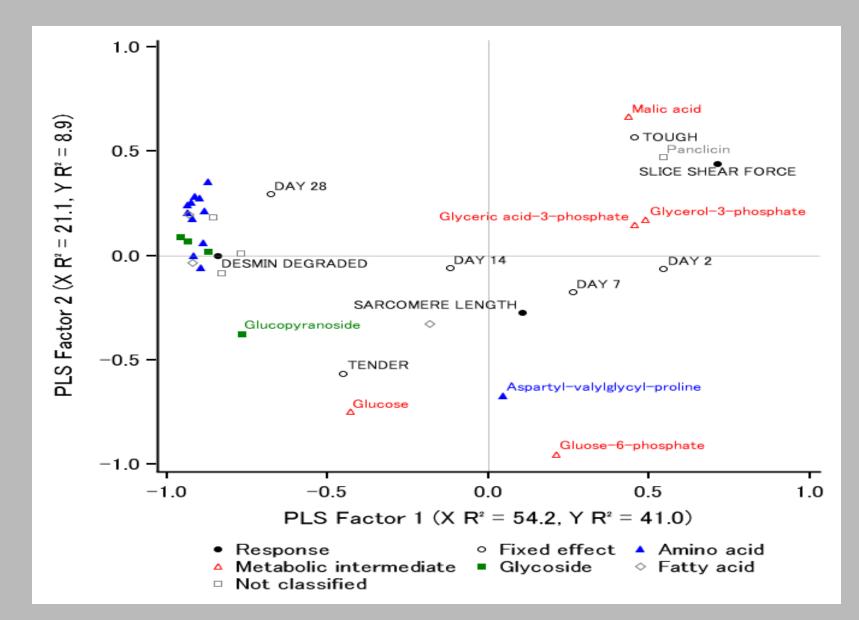
	Pearson correlation			Partial correlation (dpm removed)		
Significance level	Slice shear force	Desmin degraded	Sarcomere length	Slice shear force	Desmin degraded	Sarcomere length
P < 2 × 10 ⁻⁵	206	294	0	61	61	0
P < 0.05	437	534	55	310	259	69

• 102 selected for PLS regression based on partial correlation

Metabolites results

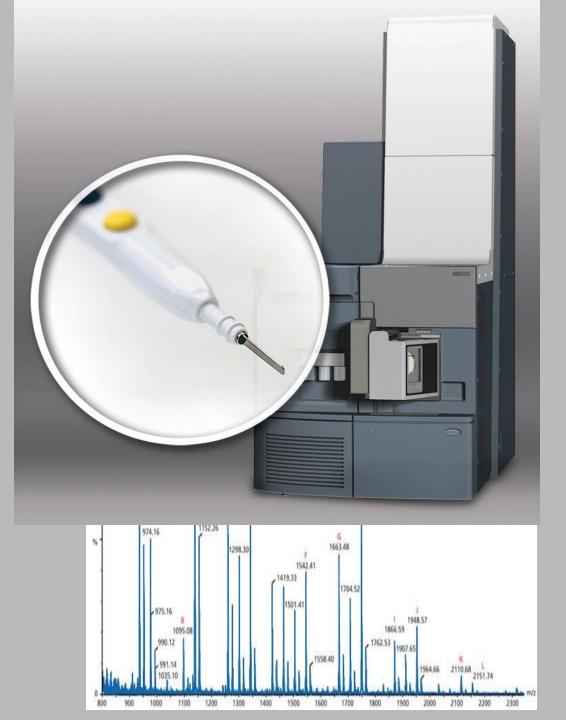
- 28 were annotated
- Free amino acids/small peptides (n = 12)
 - Greater in tender, highly associated with proteolysis
- Glycolysis/Kreb's intermediates(n = 5)
 - More glycolytic muscles produced more tender steaks
- Glycosides (n = 4)
 - Increased postmortem, associated with proteolysis, more so in steaks classified as tender
- Fatty acids (n = 2)
 - Greater increase in abundance during aging in steaks classified as tender.

Partial least squares regression



Rapid Evaporative Ionization Mass Spectrometry (REIMS)

- Originally developed for cancer research
- "Burns" and analyzes volatiles
- Gives Mass Spec fingerprint
- Compound ID??
- Real-time chemical fingerprint.
- Develop prediction equations

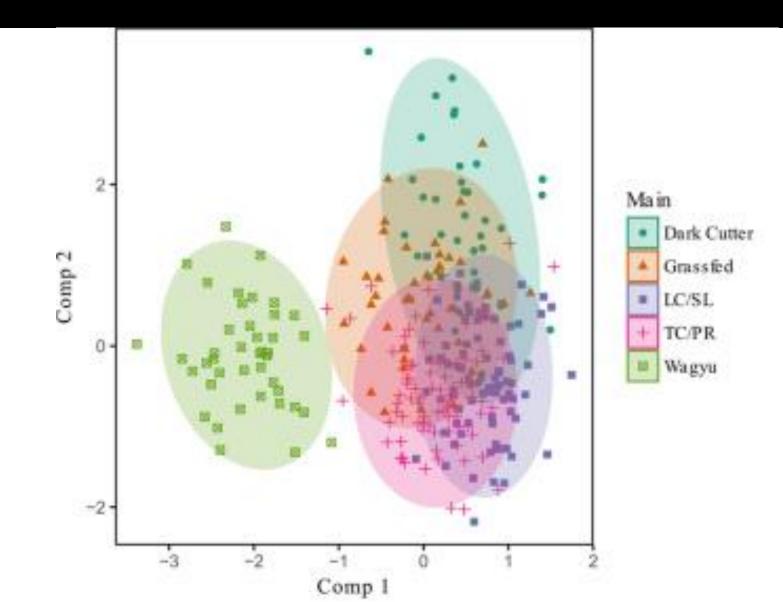


Sampling for beef REIMS experiment

Classifications (# of observations)	Model Set	S		
	Main	Specialized	Breed	Tenderness
Dark Cutter (41)	×	×		
Top Choice/Prime* (81)	×			
Low Choice/Select* (84)	×			
Wagyu (42)	×	×		
Grass fed (42)	×	×		
Tender (215)				×
Tough (74)				×
Angus (159)			×	
Not Angus (46)			×	

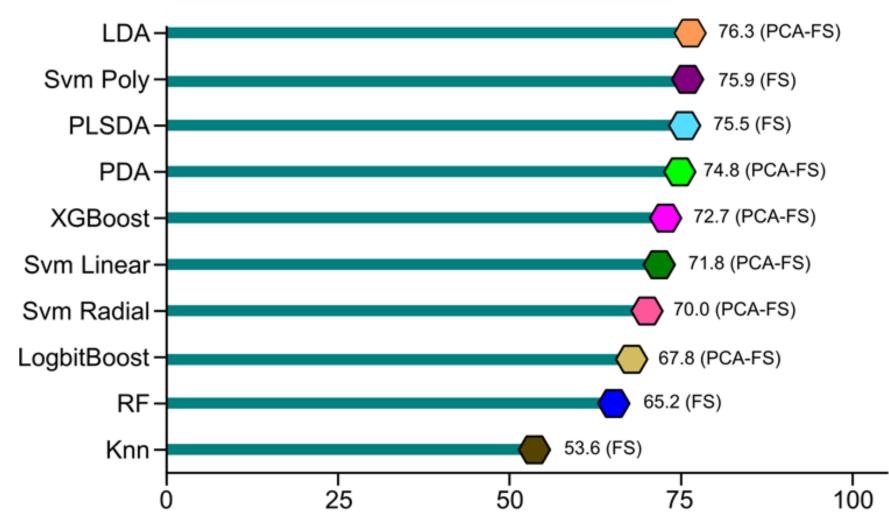
1,700 bins (compounds) detected on each sample

Main data set

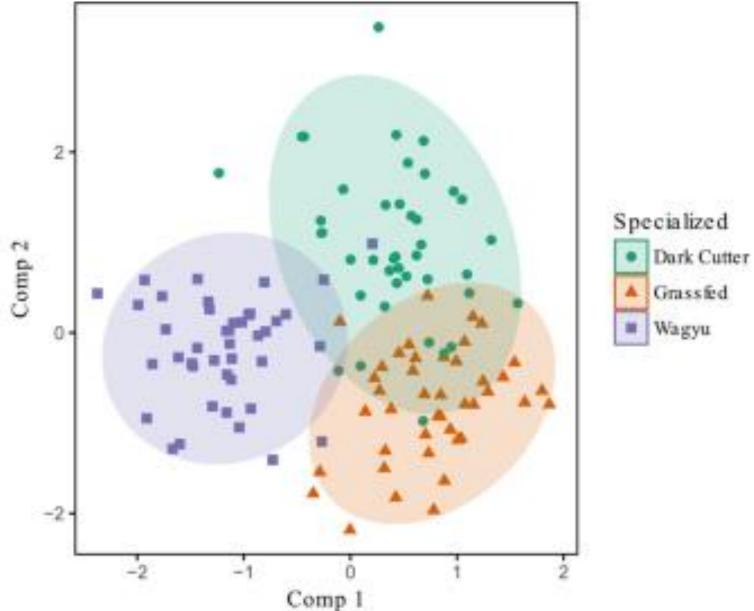


Accuracy of Main prediction

Main



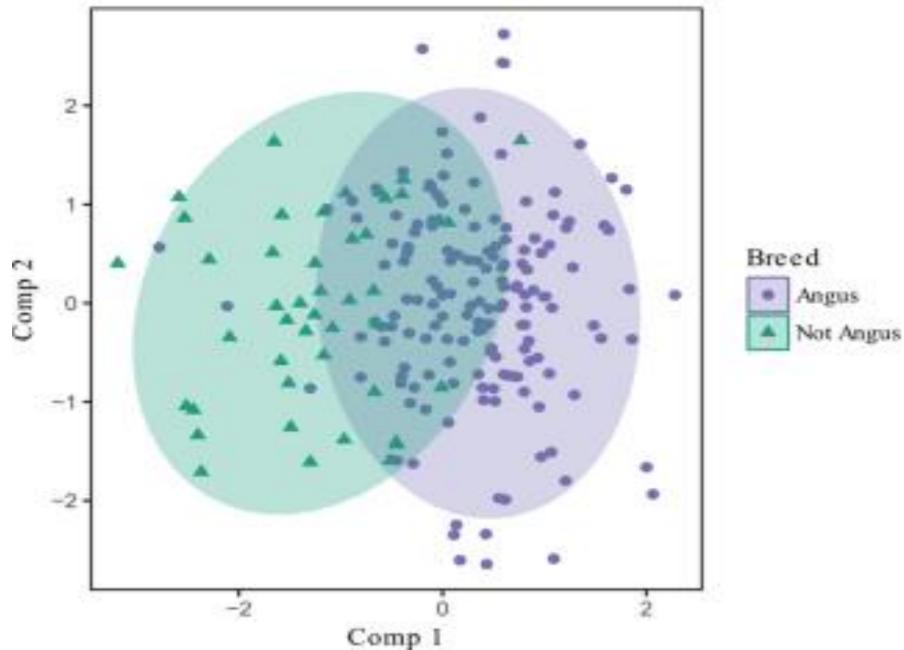
Specialized data set



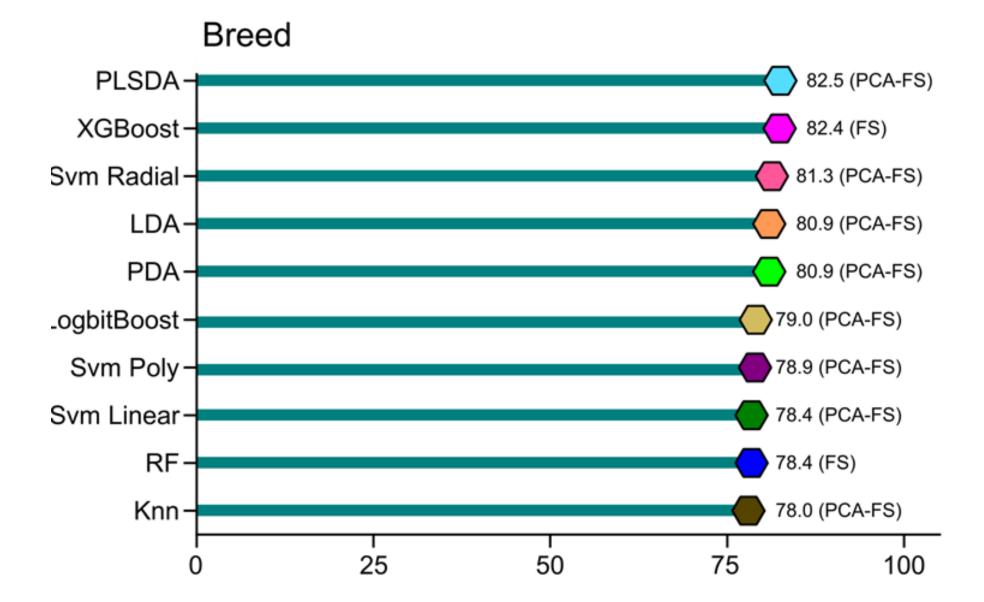
Prediction accuracy of specialized data set



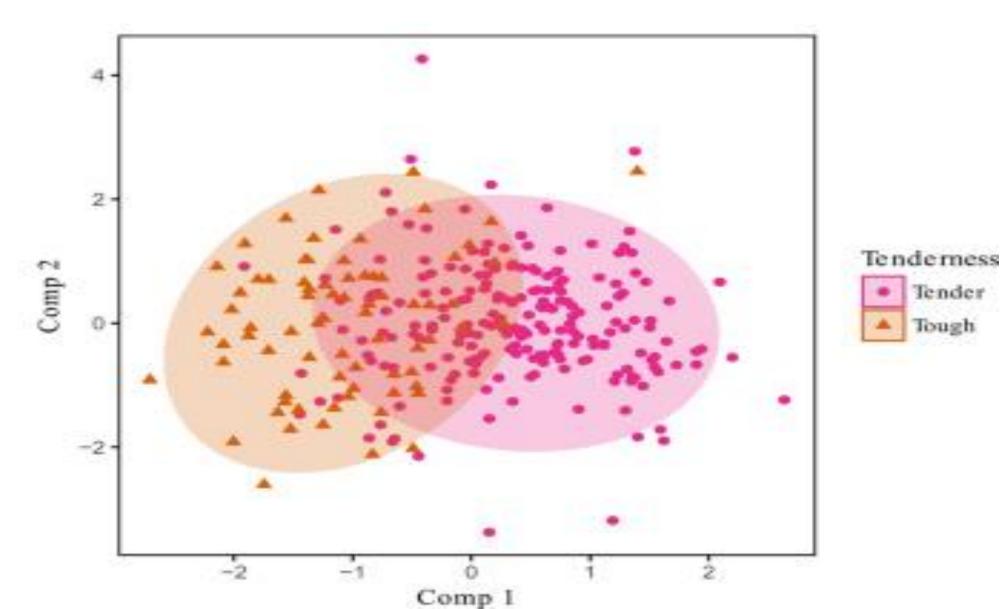
Breed type data set



Breed prediction accuracy



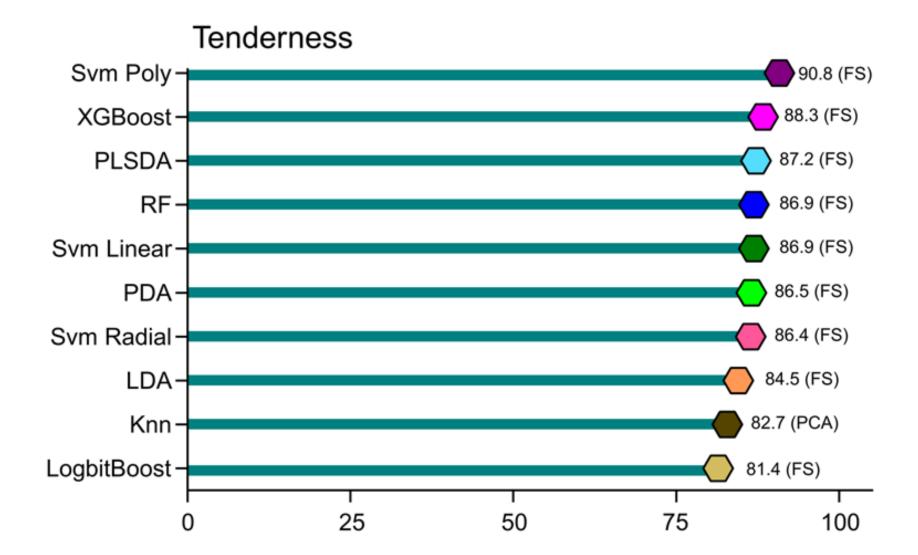
Tenderness dataset



Tender

Tough

Tenderness prediction accuracy



Final model prediction accuracies

MODEL SET	DIMENSION REDUCTION APPROACH	NUMBER OF PREDICTORS	MACHINE LEARNING ALGORITHM	FINAL ACCURACY RATE
Main	PCA-FS	24 PCs	LDA	81.5%
Specialized	FS	60 mass-bins	SVM - Linear	99%
Breed	PCA-FS	38 PCs	SVM - Radial	85%
Tenderness	FS	67 mass-bins	XGBoost	90.5%

Conclusions

- Machine learning is a growing aspect of meat science research
- Will continue to impact all parts of the meats industry
- Meat science research will increasingly be dependent on finding relationships in complex data sets.
 - Combining of multiple levels of "omics" data
- REIMS specifically has great potential in sorting beef carcasses
- With annotation could be very useful for research purposes.
- REIMS will have to be adapted to a plant environment
- Further development of models for robustness

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