New Approach to Discovery in Food Flavor

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The goal

- To chemically characterize the flavor of a food i.e. the chemical stimulants in a food delivered to the sensory systems that are responsible for flavor perception.
  - Standardization
  - Trouble shooting flavor issues – stability, quality
  - Increasing flavor intensity
  - Understanding pathways/chemistry of flavor development
  - Packaging issues

The journey

- Modern flavor chemistry is 50+ years old
- Rather young in comparison to some other fields of study

Evolution of knowledge (academic)

- 1960 - 70s – focused on the identification of all volatile compounds in foods
  - Hypothesis – if we can identify all of the aroma stimuli, we can reproduce the flavor of a food through reformulation
  - Flawed
    - Some compounds not available or not approved
    - Need quantitative data as well as qualitative
    - Wasn't that "simple" (e.g. interactions/delivery)

Concurrently – Studies on the Maillard reaction

- Identification of volatiles
- Characterization of reaction systems (process flavors)
- Identification of pathways for volatile formation

  - Could not reproduce a "process" flavor through reformulation based on volatiles

1970 → 80s Quantitative data

- Obtained some quantitative data
    - Still did not permit reconstruction of the food flavor

1970s – 1980s Statistical Relationships

- Compared gas chromatographic profiles of different food products
  - Pepsi Vs Coke
  - Wine from the north Vs south of Italy

- Could differentiate products but …. Did not have enough data to understand what was giving flavor

1970 → 1990s (present) Flavor interactions

- Strong initial focus on proteins but also work done on lipids and carbohydrates
  - Attempting to explain why different foods taste different when adding the same flavor
- Obtained a qualitative appreciation for the issue but not a quantitative
  - Little help!

1980 → present – Identification of "key" aroma compounds

- Initially – Werner Grosch and then Peter Schieberle's groups (Germany) – global effort
- Developed methods to select key compounds
  - Initially considered defining – after much work, been able to convince them this is a screening method (at best)

Select possible "Key" aroma compounds

(Semmelroch et. al., 1995).

	Activity Value	Mechanism
(E)-p-Damascenone	2.7×10^5	Carotene degrad.
2-Furfurylthiol	1.7 x l0^5	
3-Mercapto-3-methylbutylformate	3.7×10^4	
5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	1.5×10^4	Maillard reaction
Guaicol	1.7×10^3	Phenol degrad.
4-Vinylguaicol	$1.0 \text{ X } 10^3$	Phenol degrad.
Methional	1.2×10^3	Maillard reaction
2 3-Diethyl-5-methylpyrazine	**95**	

Add the selected compounds to an unflavored base (deodorized base).

- Subject the sample to sensory analysis to validate selection and optimize quantities
  - FAILED!
- How to? Select 30 compounds and put them into model system to optimize the mixture – HOW?
- 30 variables at perhaps 3 concentrations

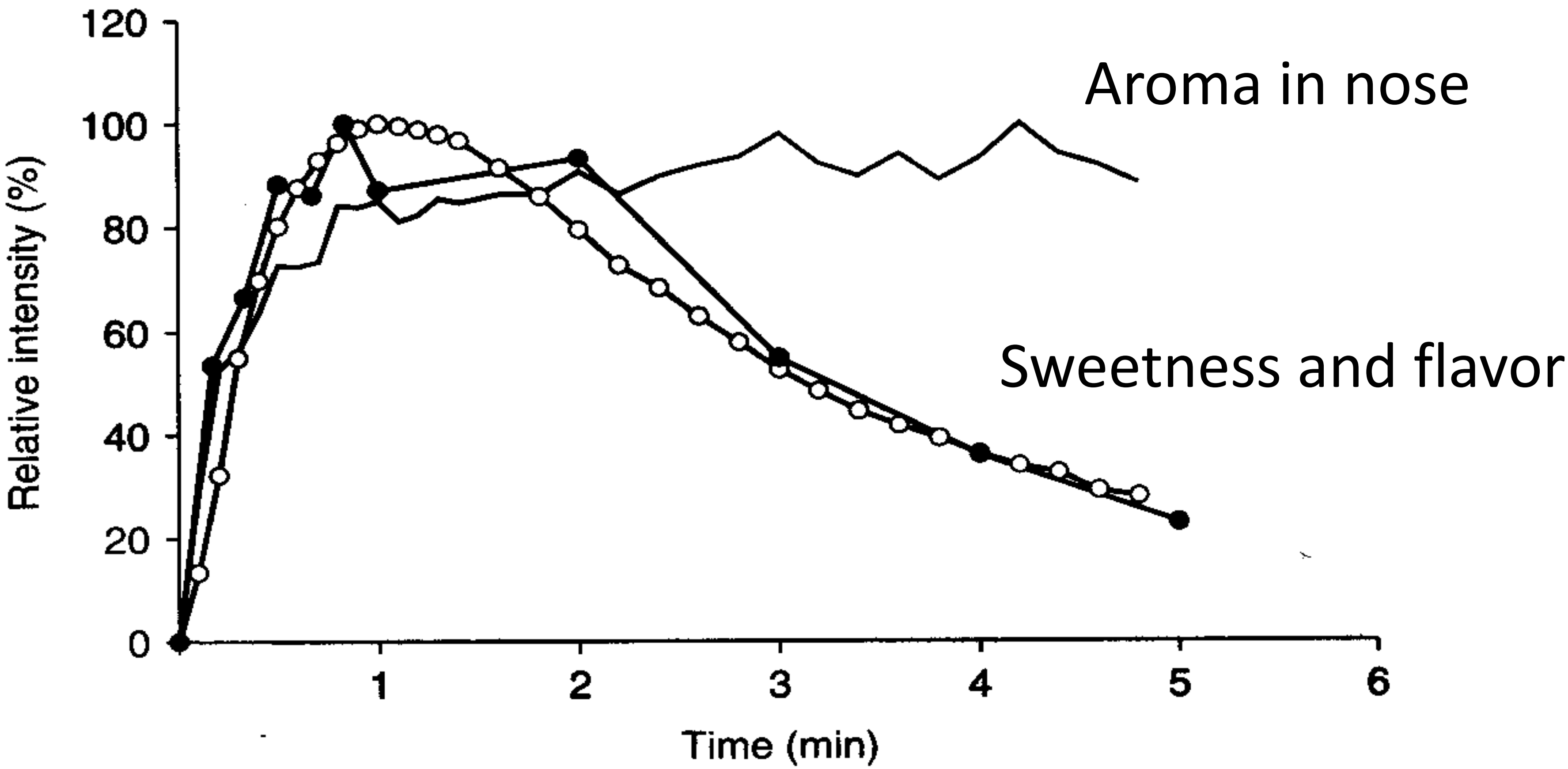
Mid 1990s – Aroma release

- Andrew Taylor et al. - in-vivo; many others developed artificial mouths

- API MS of volatiles in one's breath on eating a food; later included taste

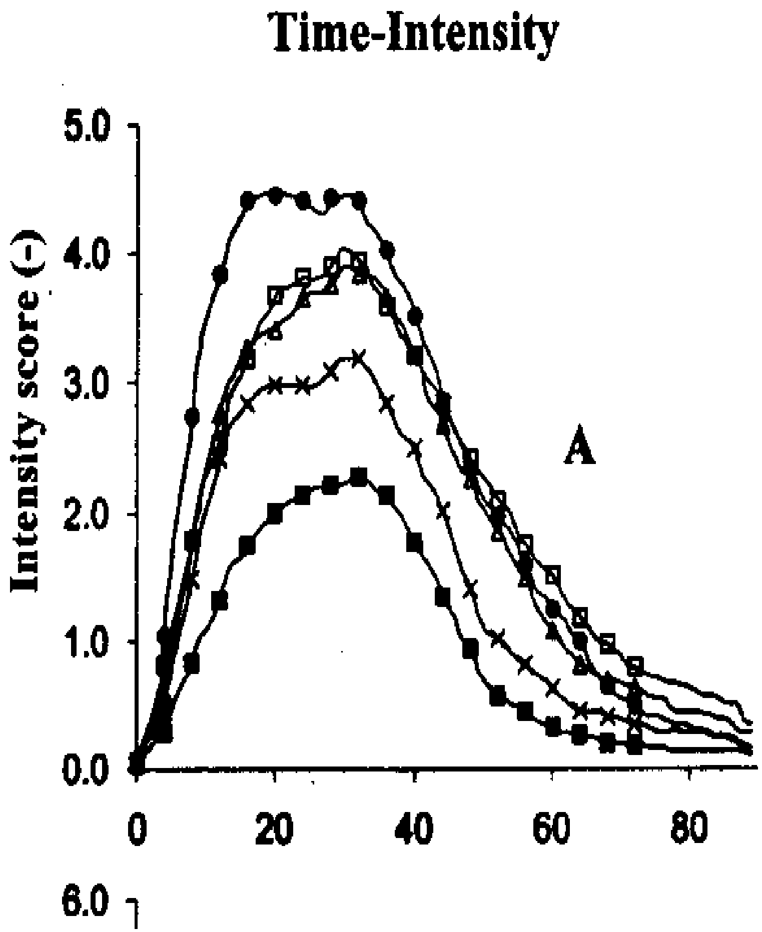
- Learnings
  - Studies on how aroma release is linked to food composition and structure
  - A start on how stimuli relates to perception

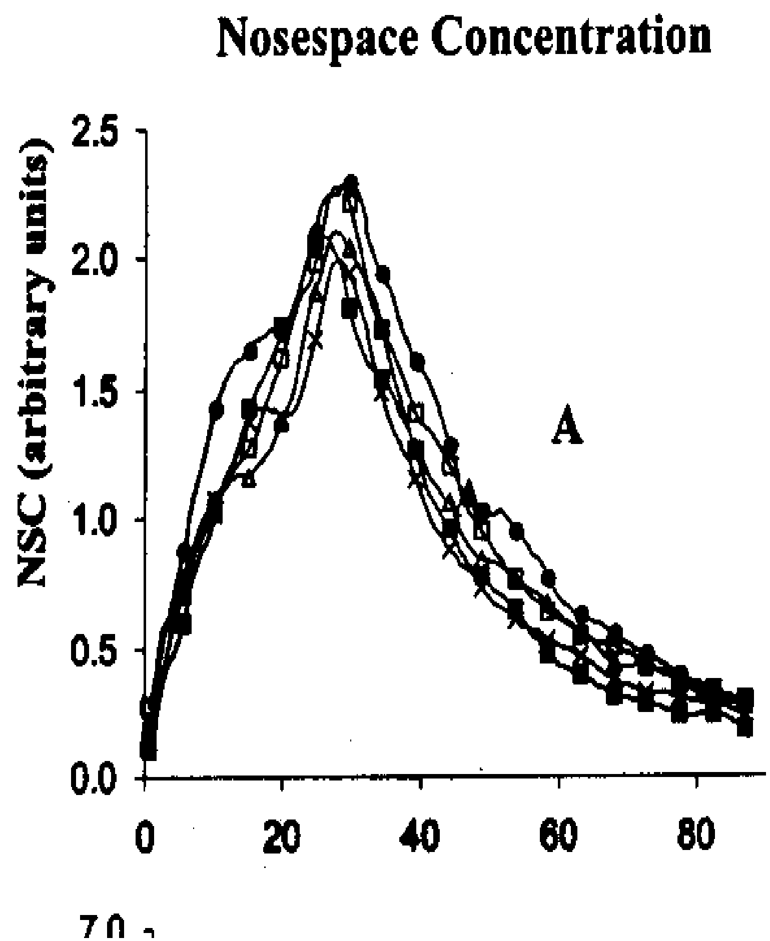
Sweetness, aroma and perception in chewing gums



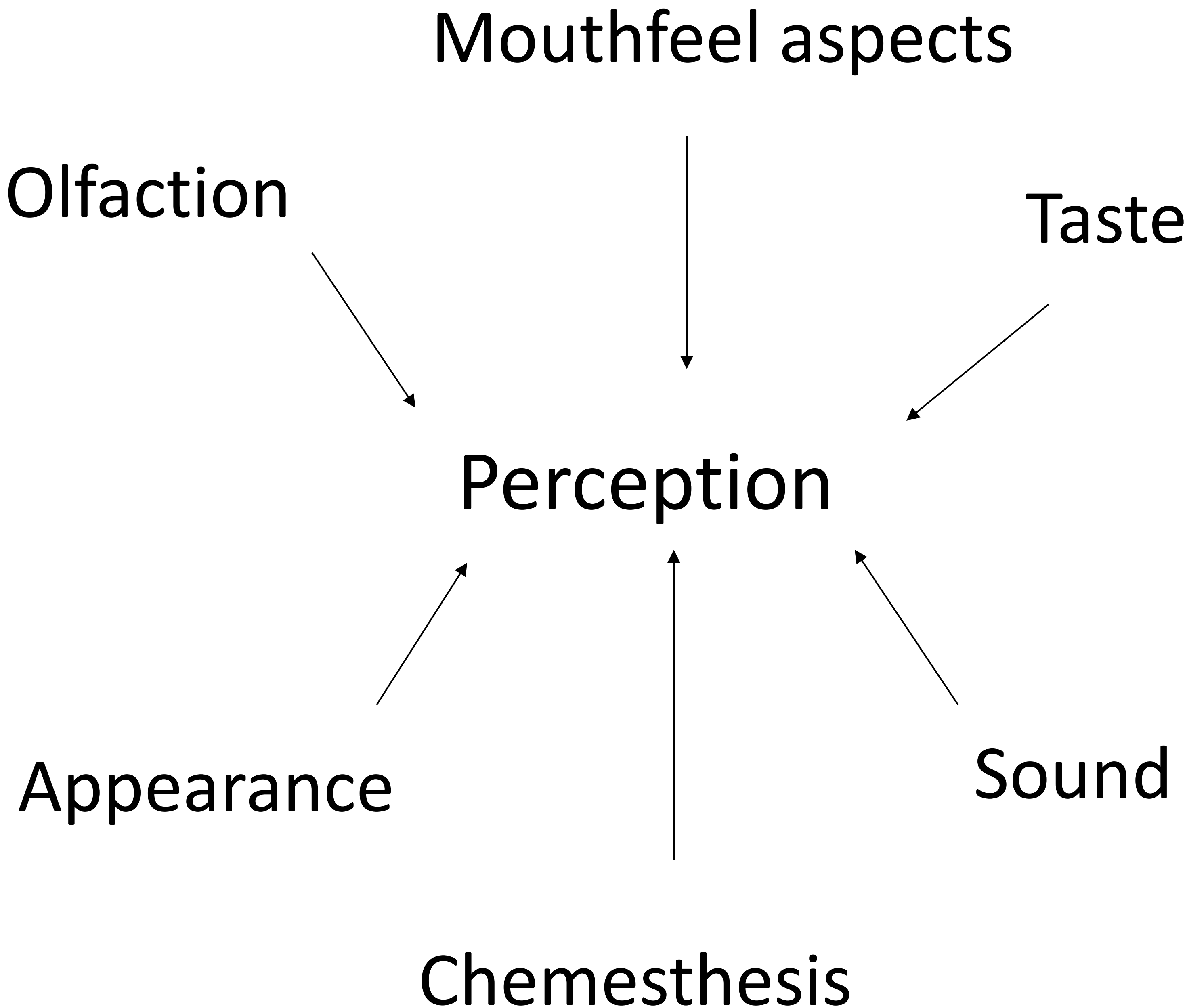
Davidson, JM, TA Hollowood, RST Linforth, AJ Taylor 1999, J Ag Fd Chem 47:4336

Effect of Whey Protein Gel Strength on Aroma Release (no sweetener)





Weel et al. 2002. J Ag Food Chem 50:5149


Mouthfeel aspects
Olfaction
Taste
Perception
Appearance
Sound
Chemesthesis

Challenge remains

- How do we take chemical data and link it to perception i.e. Chemically define the stimuli that define perception of a given flavor, in a given food matrix?
- Need a method that takes interactions and release of an extremely complex set of stimulants into consideration.

Chemometrics (Flavoromics)

- ✓ Comprehensive & ***data-driven***

Non-targeted

Flavor – inputs from all measurable chemical stimuli

ALL instrumental data collected are valuable *a priori*

(not restricted to earlier "thinking")

Unbiased view of the food system

Flavoromics

- ✓ Comprehensive & data-driven
- ✓ Multi-disciplinary
- ✓ Chemometrics

Mathematical & statistical tools used to make rationale analysis of chemical measurements

Why needed?

Large data sets with multiple variables

Data visualization & interpretation

DATA ≠ INFORMATION

Applications

FLAVOROMICS

Prediction of sensory properties (flavor)

Linking chemical stimuli to flavor perception

Discovery

Single mode interactions (olfaction – mixtures > 3 cpds take on a new character)

Cross modal interactions

Contribute to perception without having flavor itself

Some challenges (Compromises)

- Broad range of compounds (physicochemical & concentration)- multiple platforms for sufficient compound coverage (sensitive and comprehensive)
- High throughput – Severe limitation
- Success of flavor prediction depends strongly on samples used – sample needs
- Data handling - amounts & complexity of data generated

Flavoromics & mandarin fruits

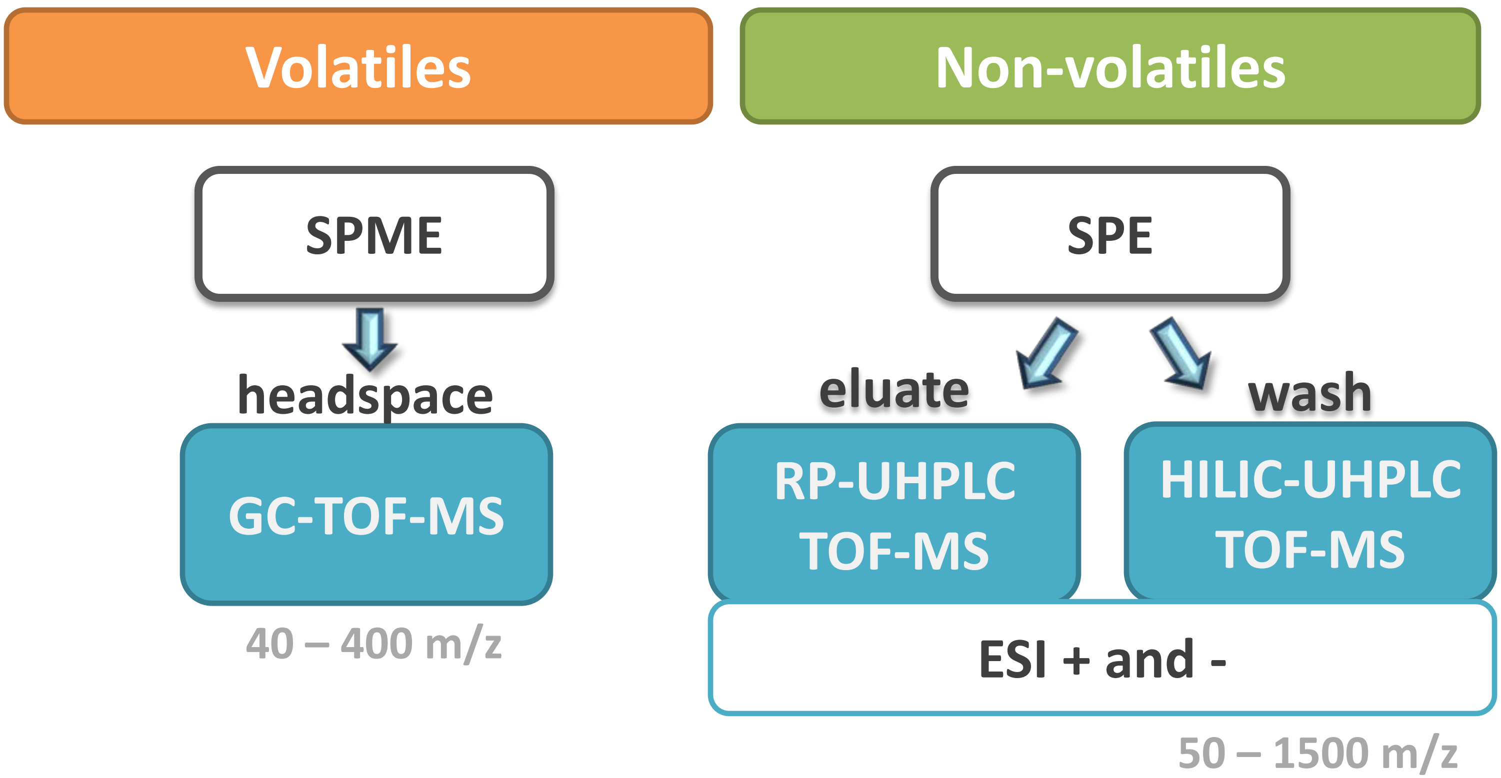
- Characterization of sensory profile of new hybrids
  - trained panel

- Identification of "flavor markers"
  - earlier selection of fruits with "potential"
  - knowledge of inheritance mechanisms of related genes

http://cambreenotes.com/wp-content/uploads/2010/02/tangerines.jpg

Non-targeted analysis of chemical stimuli

MS-based & complementary platforms



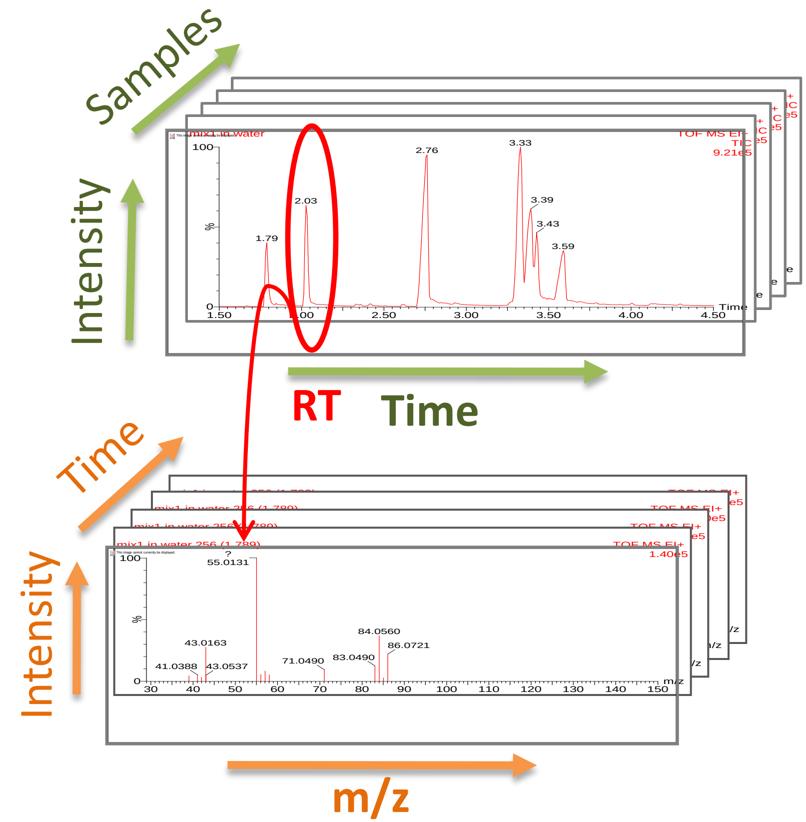
Methods developed & evaluated with a blend of orange juices

PRE-PROCESSING

- **Collected data (GC & UHPLC-MS)**
- **Variable extraction (RT – m/z)**
- **Alignment, noise subtraction**
- **Filtering, normalization**
- **Variable reduction**
- **Averaging, centering, scaling**

 # variables

GC: 134 (9420)
RP neg: 439 (1116)
RP pos: 309 (1143)
Amide neg: 300 (1002)
Amide pos: 255 (1249)



	RT- m/z			
	2.0210 - 146.0591	….	6.9022- 425.1216	7.0587 - 373.1280
Juice A	**Normalized relative intensities**			
….				
Juice D				

Adapted from Boccard et al. J Sep Sci. 2010, 33: 290-304; Chen et al. Drug Metabol. Rev. 2007, 39: 581–597.

Project workflow

Mandarin juices differing in flavor characteristics

Instrumental analyses

Chemical stimuli

Descriptive sensory analysis

Flavor attributes

Descriptive sensory analysis

Recruit panel

Generate lexicon

Evaluate judges & lexicon

Sample evaluation

Sensory data matrix

13-15 subjects

USDA/ARS, Citrus and Subtropical Products Laboratory (FL)

Training (~ 20 h each year)

19 descriptors (aroma & taste)

Sensory test (over 2 years)

	Green	Orange	Floral	…
Sample 1	**Panel average scores**			
….				

Project workflow

Mandarin juices differing in flavor characteristics

Instrumental analyses

Descriptive sensory analysis

Chemical stimuli

?

Flavor attributes

Predictive models (PLSR)

Partial Least Squares regression – 1

Modeling method that finds a linear multivariate model to link two data matrices

INSTRUMENTAL

	RT- m/z	RT- m/z		
Juice 1				
…				
Juice i				

X

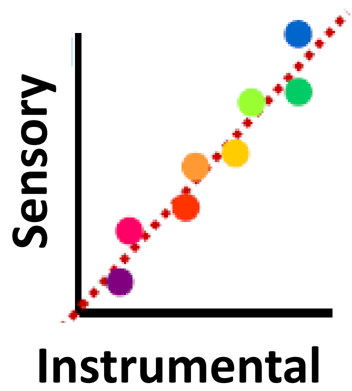
Predictors

SENSORY

	citrus	floral	bitter	….
Juice 1				
…				
Juice i				

Y

Responses



Find directions in X which are predictive of directions in Y

Maximizes correlation

CORRELATION ≠ CAUSATION

PLSR – model selection 2

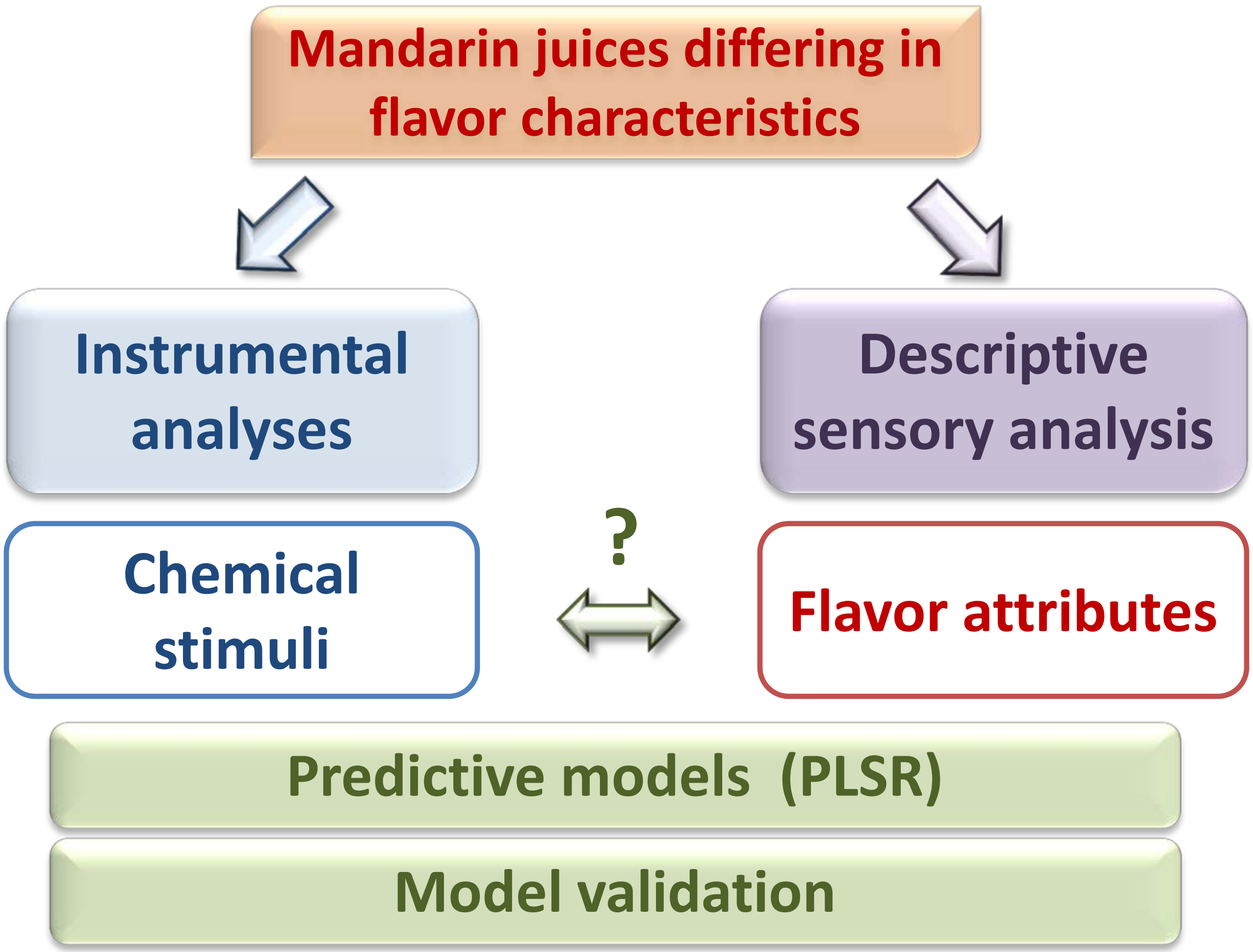
- Improved explanatory & predictive performances of the models when merging all instrumental data into one single data set (as opposed to individual ones)

- Best model with combined data & variable selection
  - 576 instrumental variables

Combined data & variable selection

GC			RP +			RP -			Amide +			Amide -		
	…	Var_j		…	Var_j		…	Var_j		…	Var_j		…	Var_j
Juice 1			Juice 1			Juice 1			Juice 1			Juice 1		
…			…			…			…			…		
Juice i			Juice i			Juice i			Juice i			Juice i		

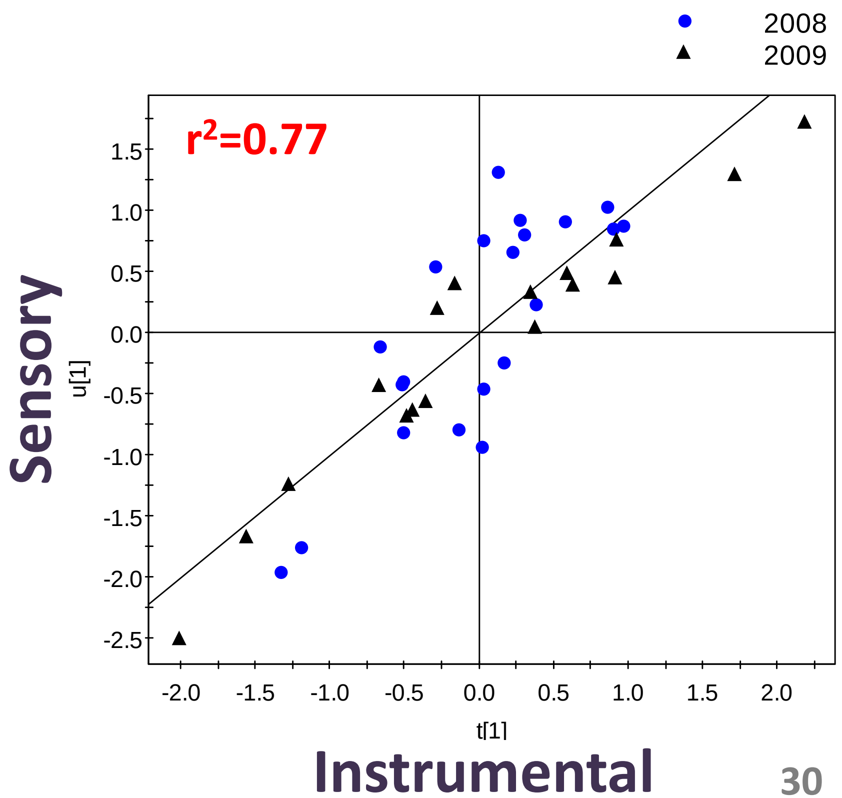
Project workflow



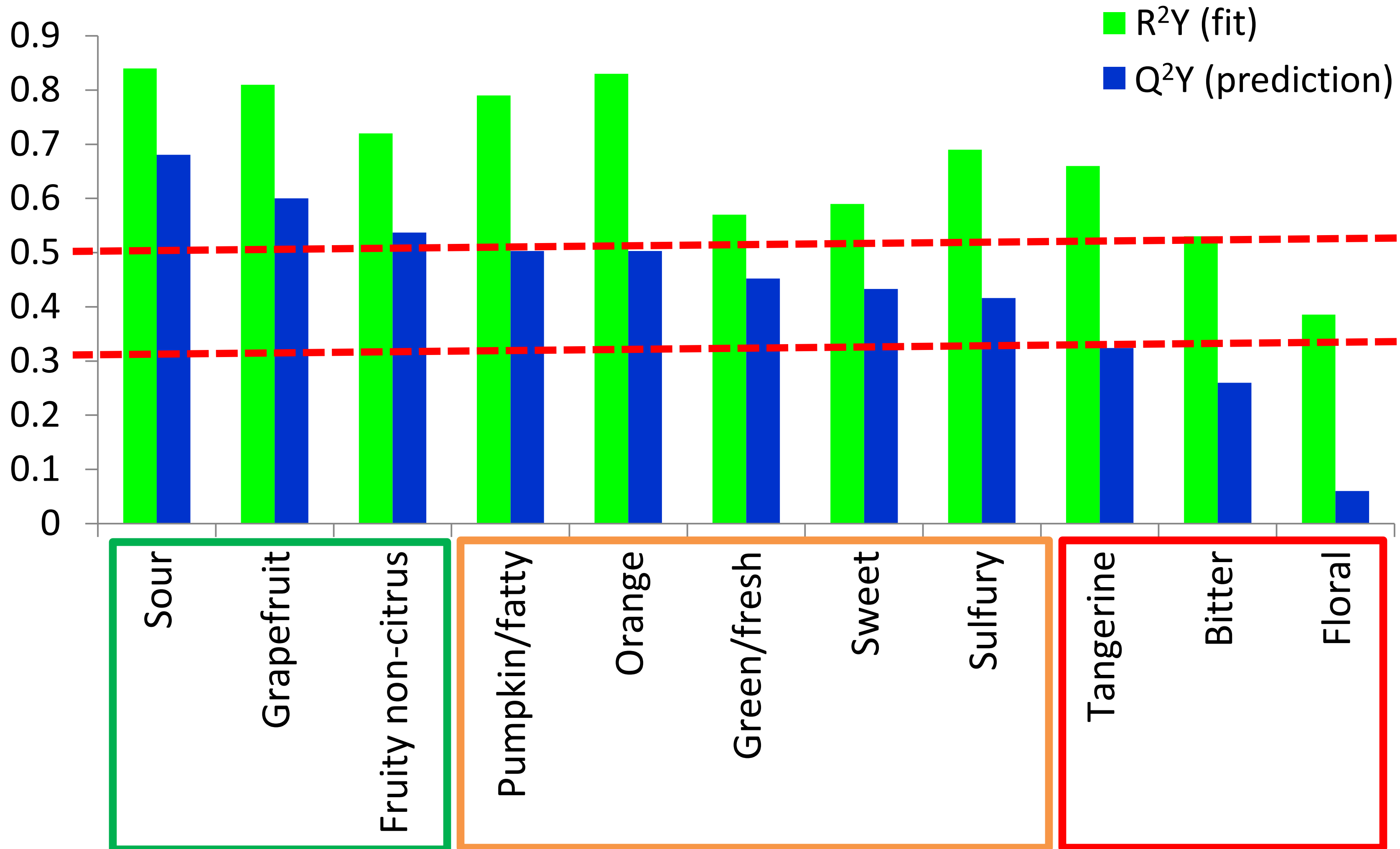
PLSR – model validation 1

- External validation
  - **calibration set** for model development (38 juices)
  - **prediction set** for model testing (8 juices)

Strong relationship between instrumental & sensory



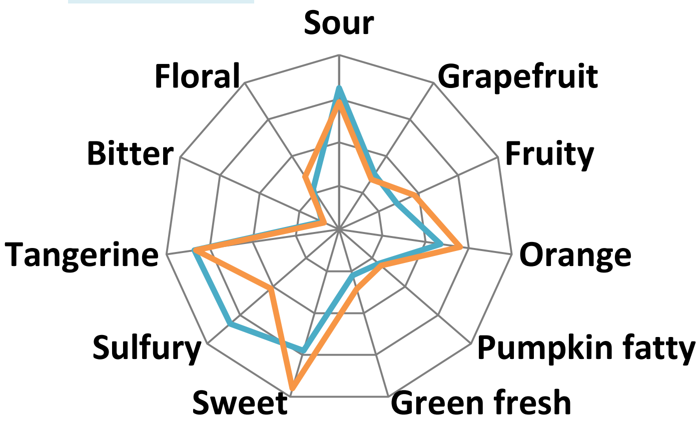
PLSR – model validation 2



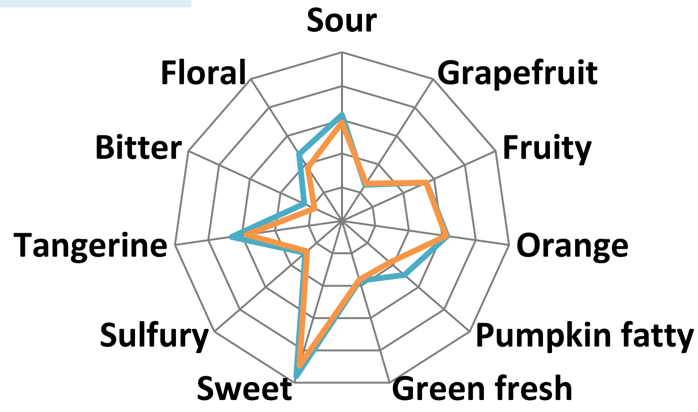
PLSR – model validation 3

Sensory profile by trained panel   Predicted by PLS model

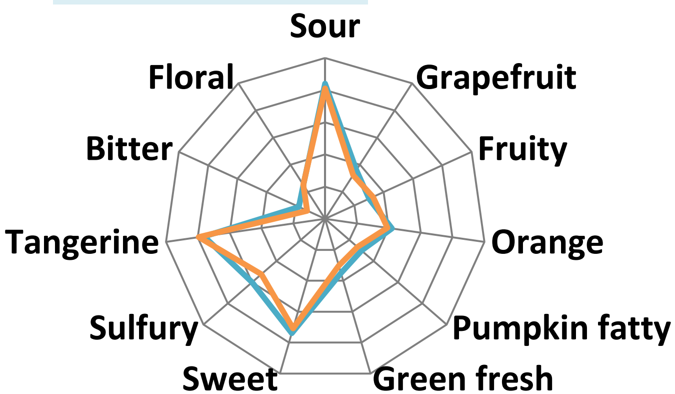
Minneola



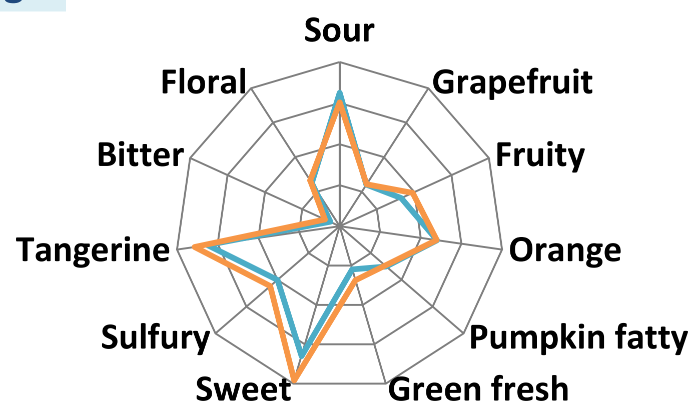
9-4 x Blood4x



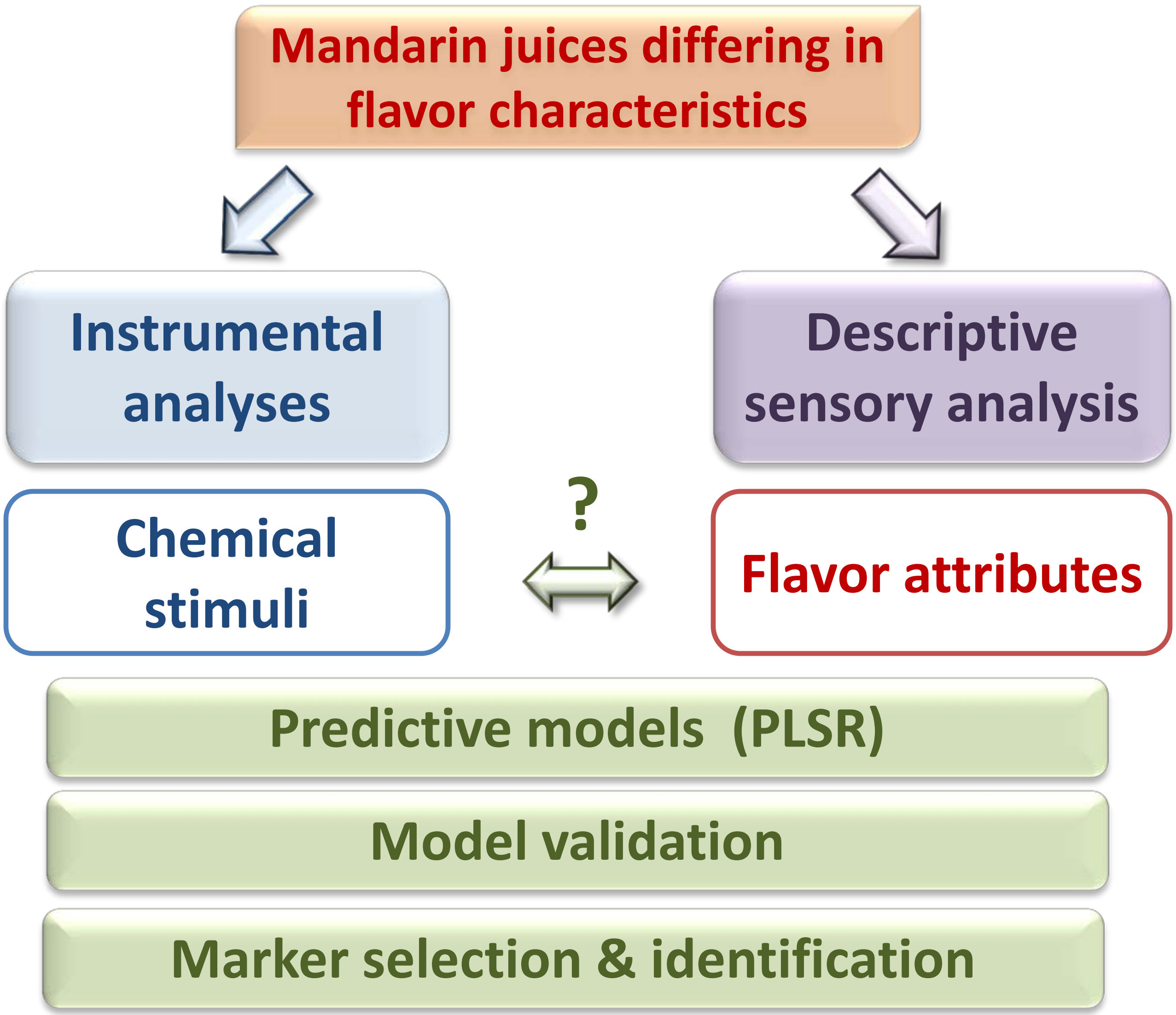
8-9 x Murcott (d)



Fallglo



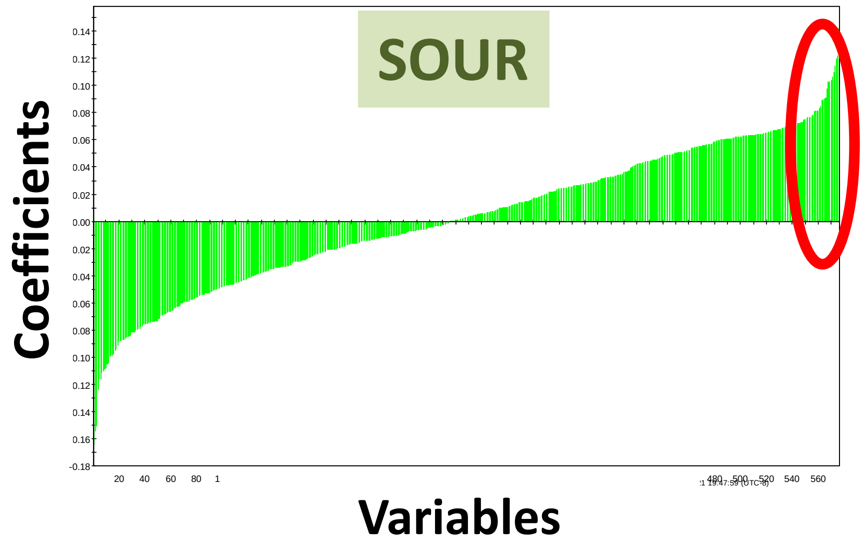
Project workflow



“Flavor marker” selection

Flavor marker = variable (compounds) most influencing the prediction of some sensory descriptors

- Regression coefficients
  - amplitude & direction of relationship of variables with selected response
  - variables w/ high & positive regression coefficient



Strategy for marker chemical identification

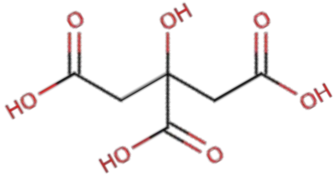
RT – m/z ???

- Mass spectra library & retention time indices
- Metabolite databases (accurate mass)
- MS/MS analyses
- Injection of authentic standard (if available)

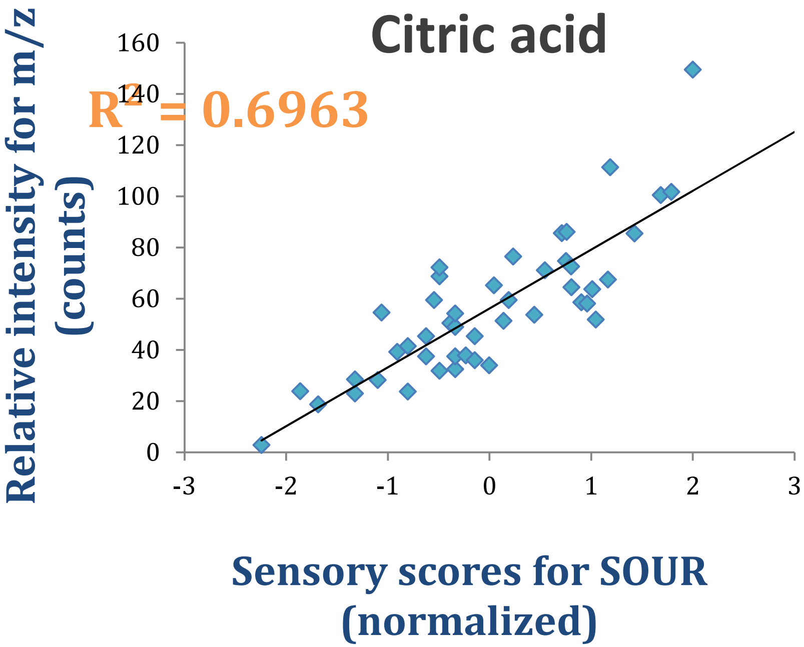
Markers identification - 1

Sour

An_6.2338_191.0167 : Citric acid



Univariate marker



Markers identification - 2

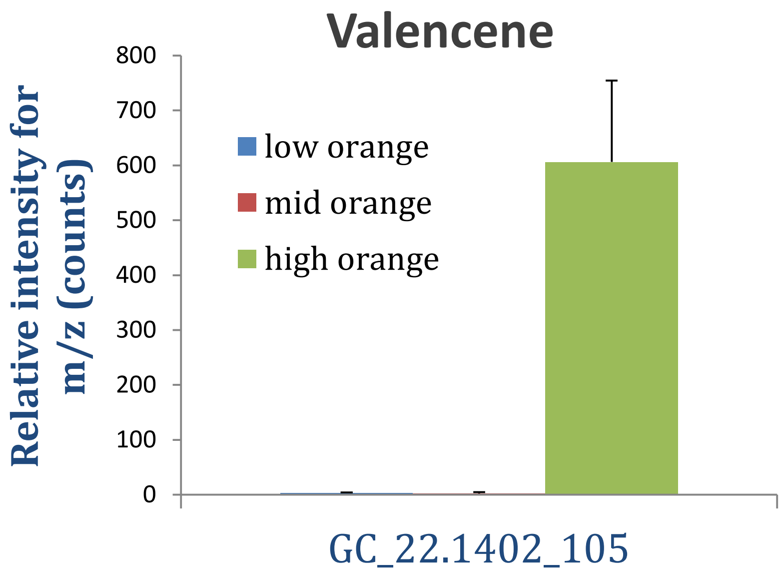
Orange

Ethyl butanoate

Sesquiterpenes

(Selinene, Valencene, α-Panasinsene)

Multivariate marker



However …

- Correlations established are NOT causative
  - studies are necessary to confirm & to understand better the nature/mechanisms of their contribution to flavor
  - Critical evaluation – selection of likely candidates

- Reported markers might have :
  - have synergetic/masking effects with other compounds

Challenges

- Much room for improvement
  - Instrumental
    - **Sensitivity (all chemical composition data needed?), MS drift, accurate quantification, problematic identification, determination of importance (?)**
  - data processing
    - Used linear models (other approaches – Random Forest or? – Ian Ronnigen)
  - Sensory
    - Difficult to separate liking from scores, dumping effect, individual sensitivity to stimulus, trained panel
    - **need for high throughout**

What do we gain?

- Improvement in flavor quality – incremental
- Definition of less characterizable attributes e.g. freshness
- Long term - definition of product flavor and acceptable and unacceptable profiles
- Drivers of individual sensory attributes – may link to plant sources, processing and storage (process flavorings)
  - Pathways and therefore methods to potentially control flavor attributes.

What cost?

- Much
  - Special room for instrumentation (humidity, temperature and “noise” control.)
  - Investment in state of the art equipment and columns
  - Multidisciplinary people – talented and dedicated!
- Use
  - FREC – Three dedicated projects in this area
  - Being applied in two major food/flavor corporations .