

Chemical and Microbially Induced Flavor Changes in Thermally Processed Citrus Juices



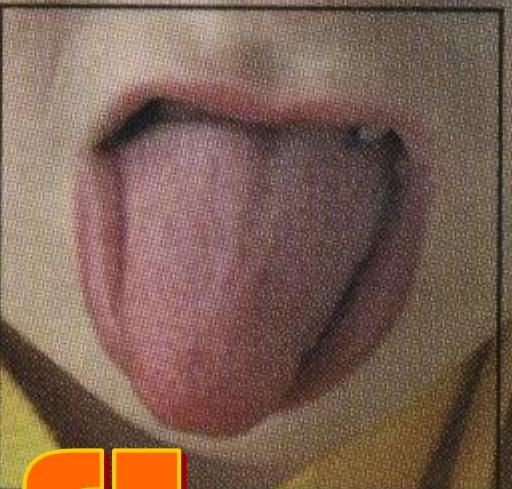
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Citrus Research & Edu. Center, University of Florida

Citrus Research Institute, Southwest University, China

Why heat citrus juices?

- Most economical to reduce microbial populations
- Also inactivates certain enzymes but ...
 - induces chemical reactions
- Acid catalyzed reactions – unsaturated terpenes
- Maillard reaction – complex mixture of products
- Thermal decompositions/reactions
 - produce sulfur volatiles

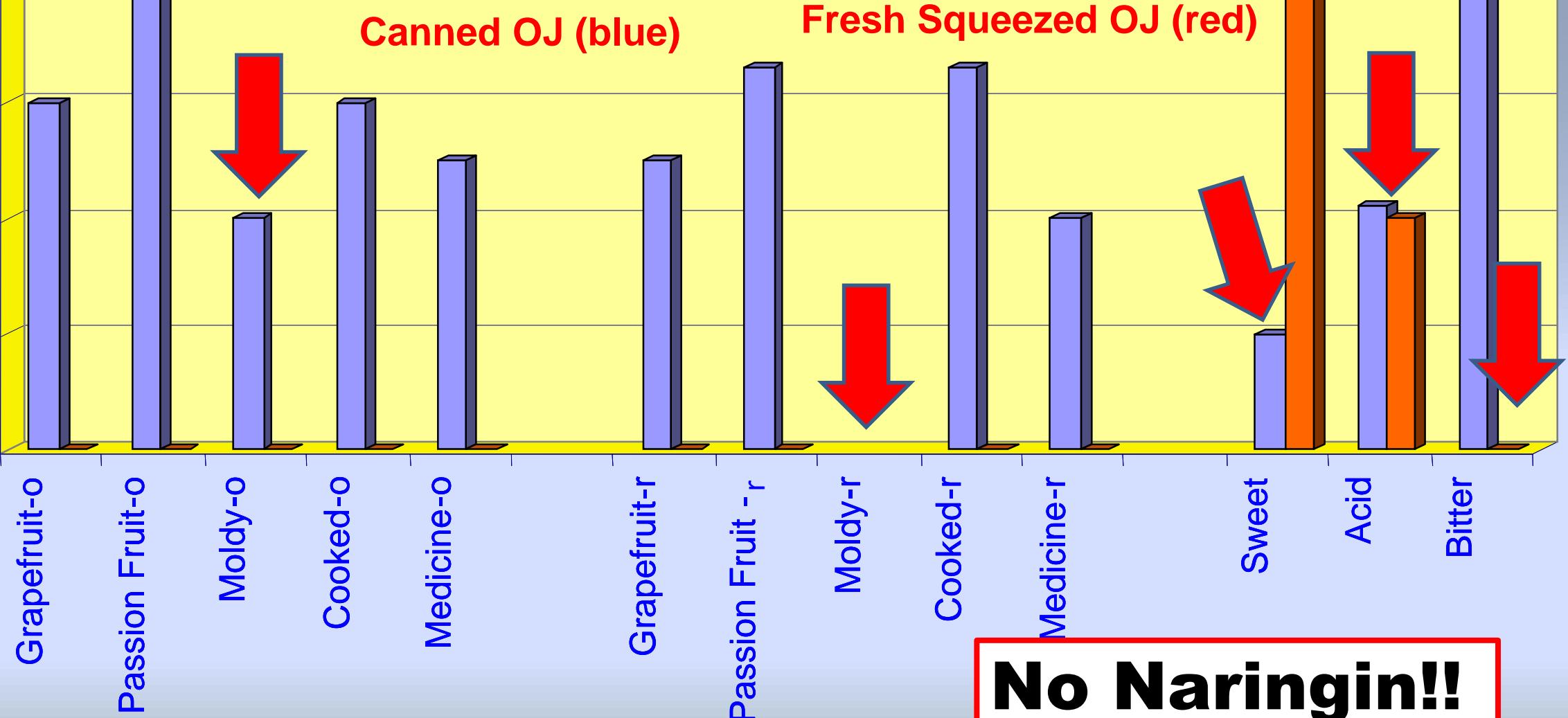


Off-flavors

Thermally Induced Chemical Changes

- Formation of sulfur volatiles – degrades flavor
 - dimethyl sulfide
 - methanethiol
 - hydrogen sulfide
- Non sulfur volatiles formed
 - 4-vinyl guaiacol (ferulic acid)
 - alpha-terpineol (limonene)
 - Furaneol (sugars + ascorbic acid)

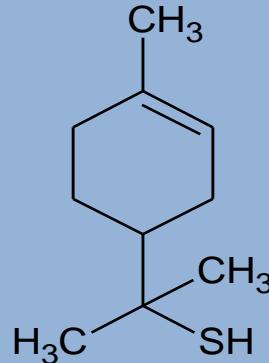
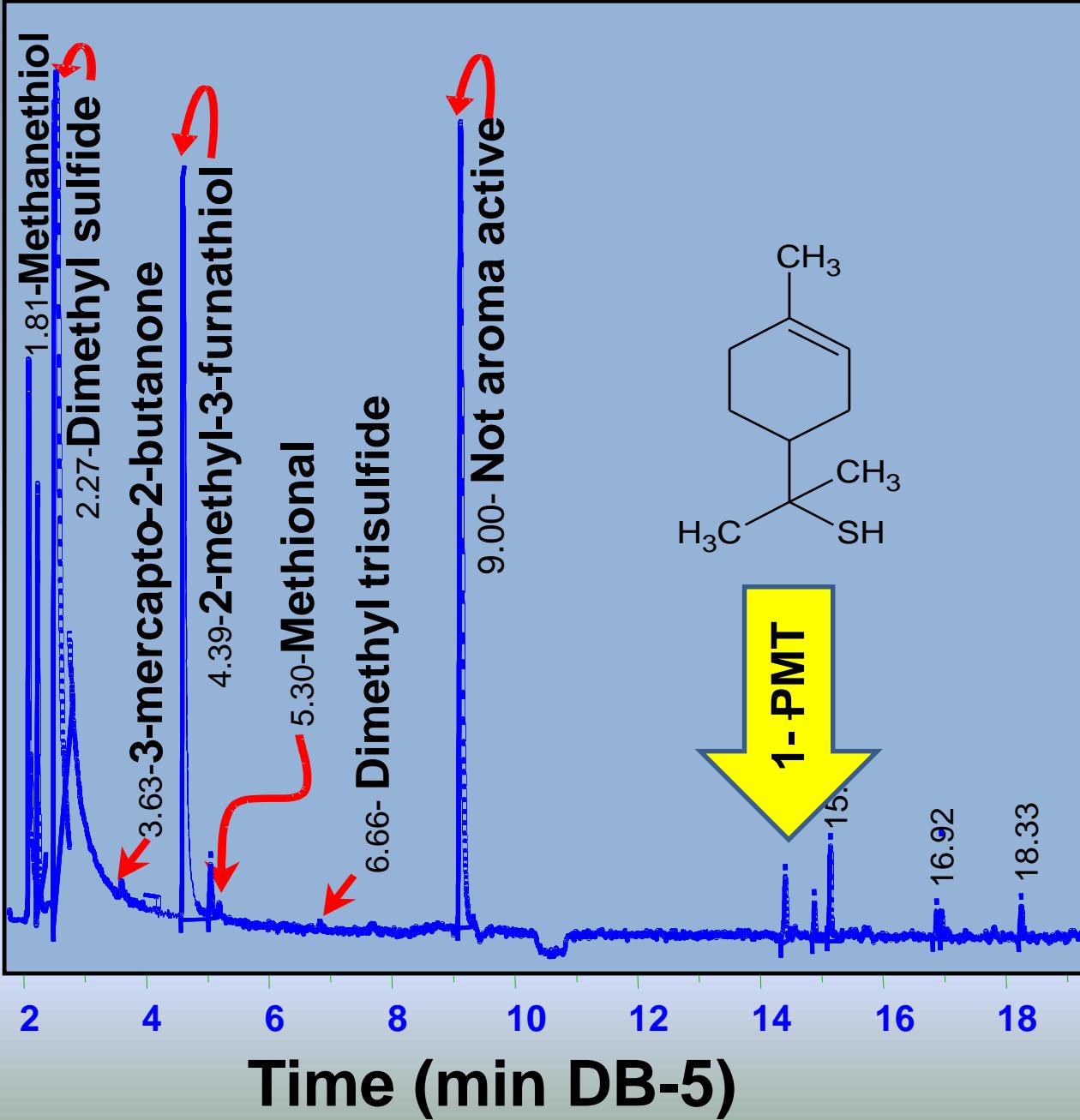
OJ without Orange Flavor



No Naringin!!

Sensory Panel Descriptors

PFPD Response



Pérez-Cacho, P. R.;
Mahattanatawee, K.; Smoot, J.
M.; Rouseff, R., Identification of
sulfur volatiles in canned
orange juices lacking orange
flavor. *Journal of Agricultural
and Food Chemistry* 2007, 55,
5761-5767.

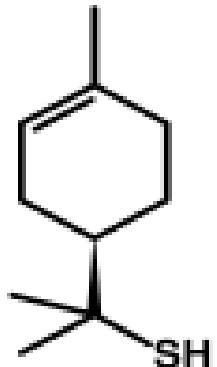
Sulfur Volatiles

- Widely distributed in Foods
- Are found in many chemical forms
- Typically found at trace concentrations
- Have extremely potent aromas
- Are difficult (BUT IMPORTANT) to measure
 - GC-olfactometry
 - Sulfur specific GC detectors - PFPD

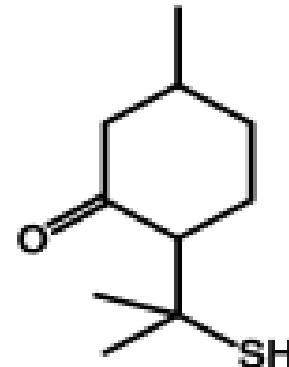
Sulfur in Cooked Foods

<i>Character impact compound (s)</i>	<i>Odor description</i>	<i>Occurrence</i>
2-Furfurylthiol	fresh brewed coffee	coffee
	coffee-like, sulfury	hazelnut, roasted
	roast-coffee	wine (barrel-aged)
5-Methylfurfurylthiol	meaty	coffee
2-Thienylthiol	coffee-like, sulfury	hazelnut, roasted
Furfuryl methyl disulfide	mocha coffee	coffee
2-Methyl-3-furanthiol	cooked meat	wine (barrel-aged)
	meaty, nutty	beer, lager
	rhubarb, fruity	beer, lager
3-Mercaptohexanol	onion, sulfur	beer, lager
2-Mercapto-3-methylbutanol		
3-Mercapto-3-methylbutanol	broth, onion, sweat	beer, lager
2-Acetyl-2-thiazoline	roasty, popcorn	potato chip
5-Acetyl-2,3-dihydro-1,4-thiazine	roasty, popcorn	
3-Thiazolidineethanethiol	roasty, popcorn	Maillard model system

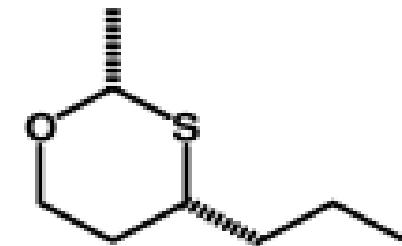
Sulfur in Fruits



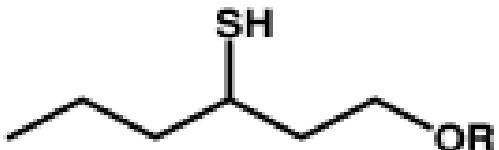
R-(+)-1-p-Menthene-8-thiol
(grapefruit)



8-Mercapto-*p*-menthan-3-one
(blackcurrant)



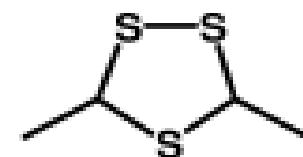
(+)-*cis*-Tropathiane
(passion fruit)



R=H 3-Mercaptohexan-1-ol
R=Ac 3-Mercaptohexyl acetate
(passion fruit, grapefruit, wine, guava)

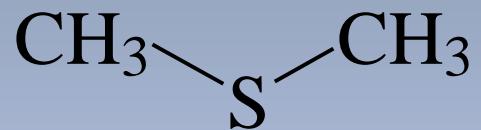


Cat ketone
(Sauvignon grape, hops,
green tea, grapefruit)

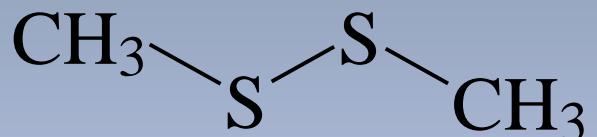


3,5-Dimethyl-1,2,4-trithiolane
(durian)

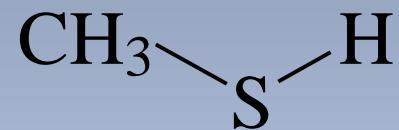
Sulfur Structures



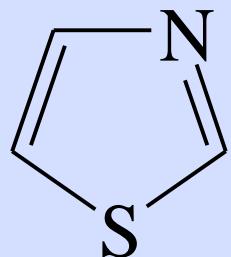
sulfide



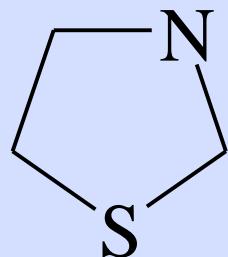
dissulfide



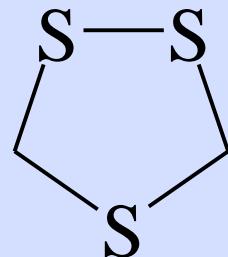
thiol



thiazole

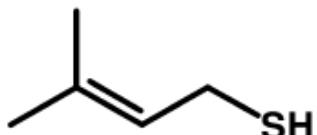


thiazolines

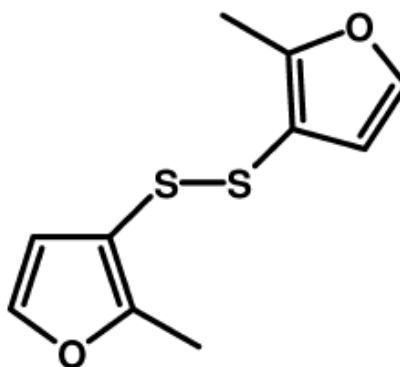


trithiolane

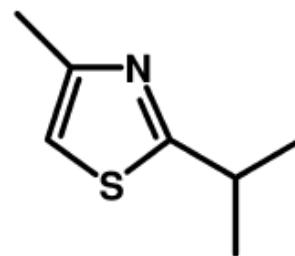
Sulfur Off-Flavor Structures



3-Methyl-2-butene-1-thiol
lightstruck beer



Bis-(2-methyl-3-furyl)disulfide
vitamin B₁ (thiamin)



4-Methyl-2-iso-propylthiazole
vitamin B₂ (fort. orange juice)

Sulfur Off-Flavors

<i>Impact compound (s)</i>	<i>Off-flavor description</i>	<i>Occurrence</i>
3-Methyl-2-butene-1-thiol	skunkly, plastic	beer (light-struck)
S-Methyl hexanethioate	cabbagy, rubbery	beer
3-Mercapto-3-methylbutyl-formate	cat urine, ribes	beer, aged
4-Mercapto-4-methyl-2-pentanone	cat urine, ribes	beer, aged
2-Mercapto-3-methylbutanol	onion-like	beer
Methional	worty cooked vegetables cooked potato potato	beer (alcohol-free) oxidized white wine orange juice, aged UHT-milk
Dimethyl disulfide	sunlight off-flavor	milk
2-Methyl-3-furanthiol	meaty/vitamin B	orange juice, aged
Benzothiazole	sulfuric, quinoline	milk powder
Bis(2-methyl-3-furyl)disulfide	vitamin B ₁ odor	thiamin degradation

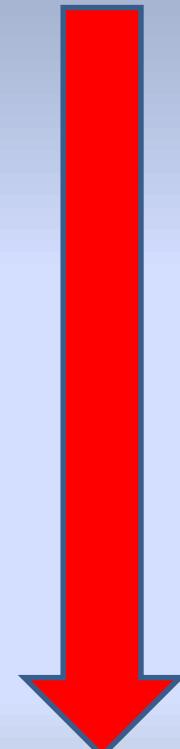
Relative Aroma Strength

thiols > heterocyclo > disulfides > sulfides

Rough rule – many exceptions

Sulfur Aroma Thresholds

<i>Compound</i>	<i>Odor Description</i>	<i>Odor Threshold ($\mu\text{g/L}$)</i>
Hydrogen sulfide	Rotten egg, sulfurous	10
Methional	Cooked potato	1.8
2-Acetylthiazole	<i>popcorn</i>	1
Methanethiol	Sulfurous	0.2
Dimethyl disulfide	cabbage	0.16
2-Acetylthiophene	sulfury	0.08
2-Furfurylthiol	Roasted, coffee like	0.005
2-Methyl-3-furanthiol	Boiled meat	0.005
Bis(2-methyl-3-furyl)-disulfide	beef like	0.00002



Human Nose Extremely Sensitive to Solvates

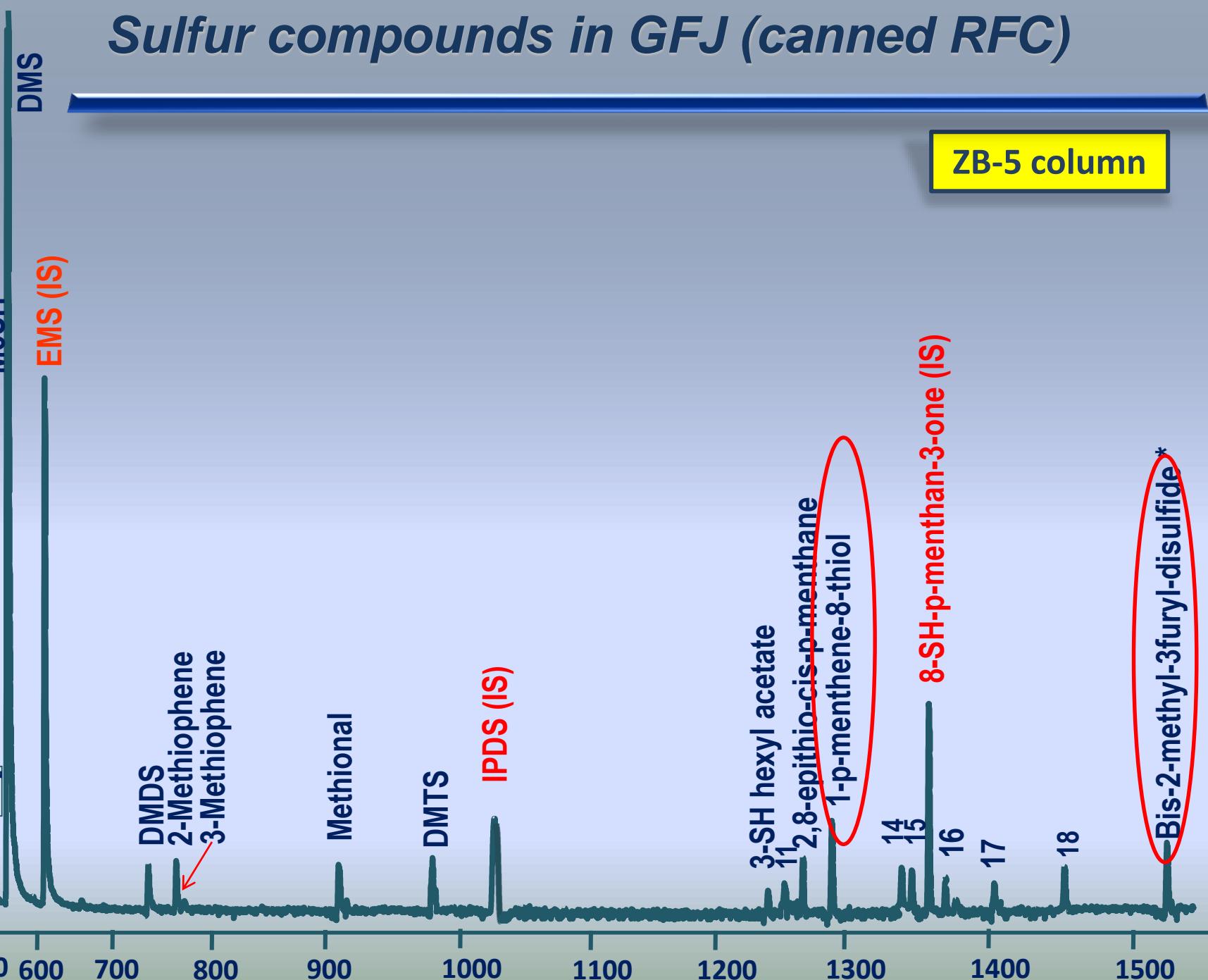
How to measure at trace levels?

Sensitive Sulfur Specific Detectors

- Atomic Emission
- Sulfur Chemilluninescence
- Flame Photometric detection
- Pulsed Flame Photometric Detection, PFPD
- Selected Ion Monitoring, SIM

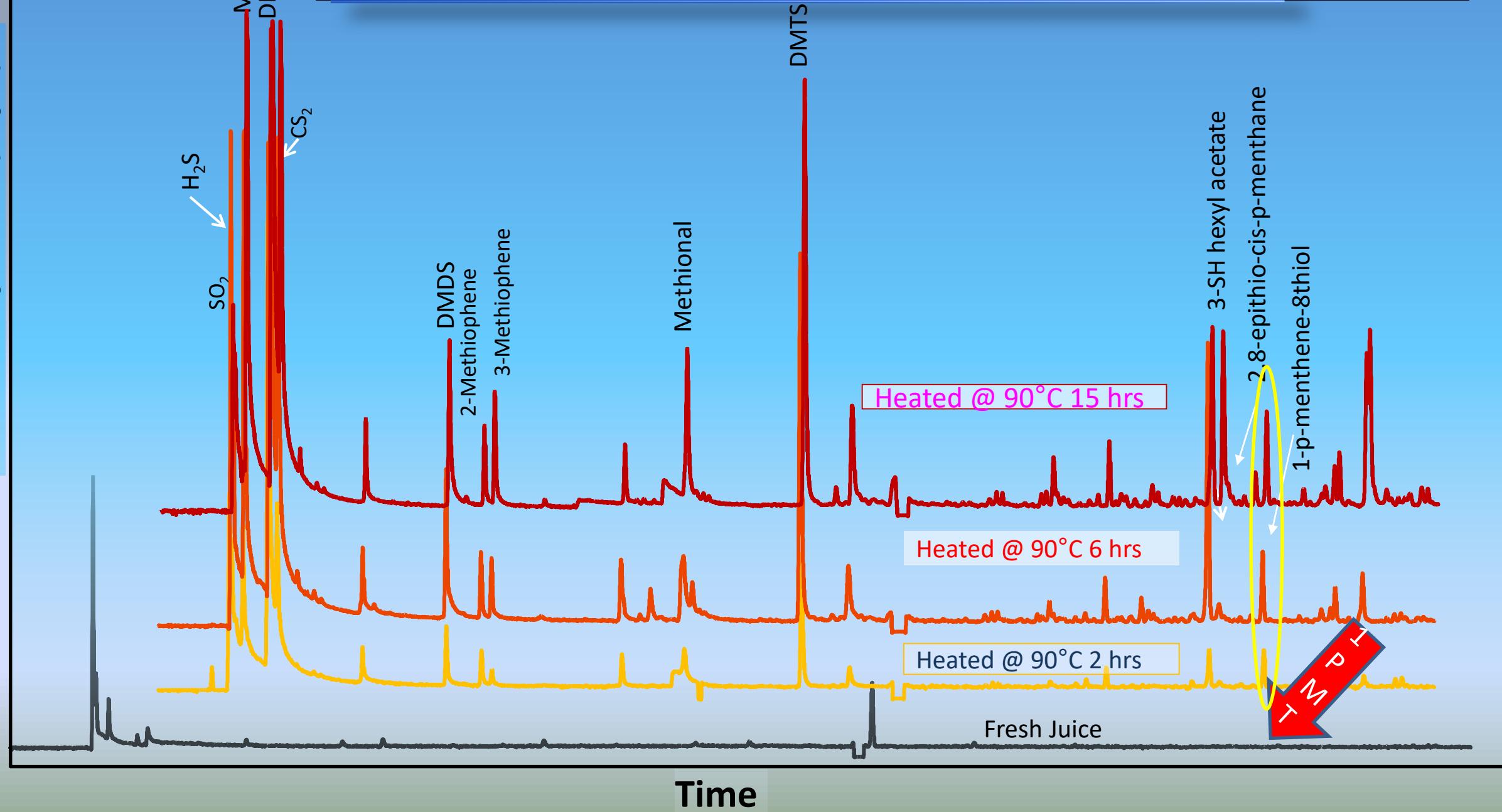
Sulfur compounds in GFJ (canned RFC)

PFPD Response (Sqrt mode)

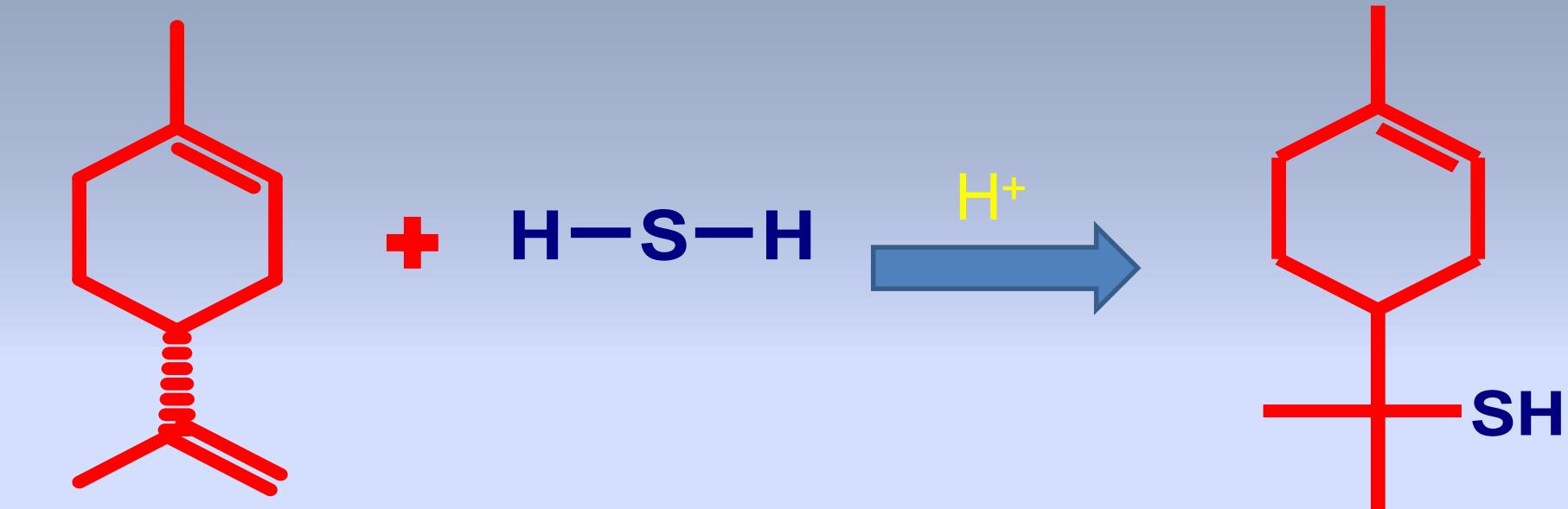


Thermal generation of GFJ VSC's

PFPD Response (Sqrt)



Mechanism for 1-PMT formation



+limonene

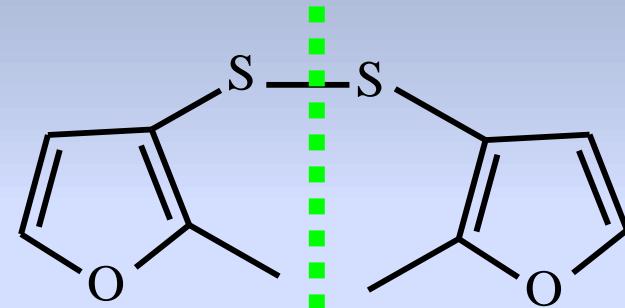
citrus

1-p-menthen-8-thiol

Grapefruit – character impact

Flavor Precursors

Thiamine Decomposition



bis (2-methyl-3-furyl)-disulfide

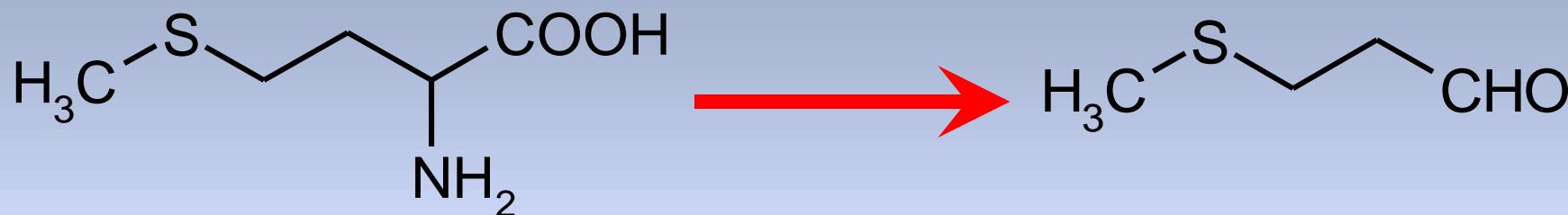
Thiamine (vitamin B1)

¹⁴

threshold = 2 parts in 10^{14} parts water

Dreher, J. G.; Rouseff, R. L.; Naim, M., GC-Olfactometric Thermal Degradation of Thiamin in Model Orange Juice. *Journal of Agricultural and Food Chemistry* 2003, 51, 3097-3102.

Sulfur Amino Acid- Strecker Aldehyde



methionine

Tasteless-odorless

methional

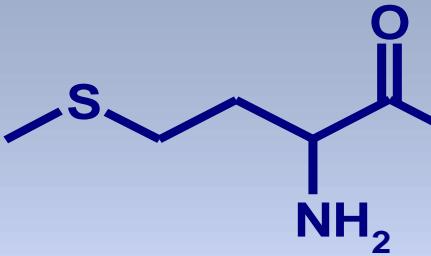
Cooked potato

Nonvolatile Precursors in Citrus

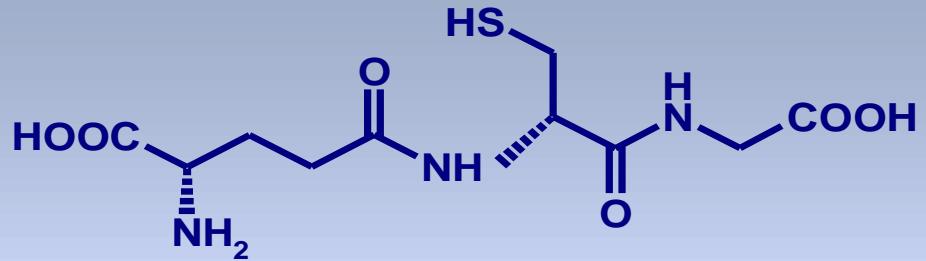
Sulfur containing amino acids



Cysteine



Methionine

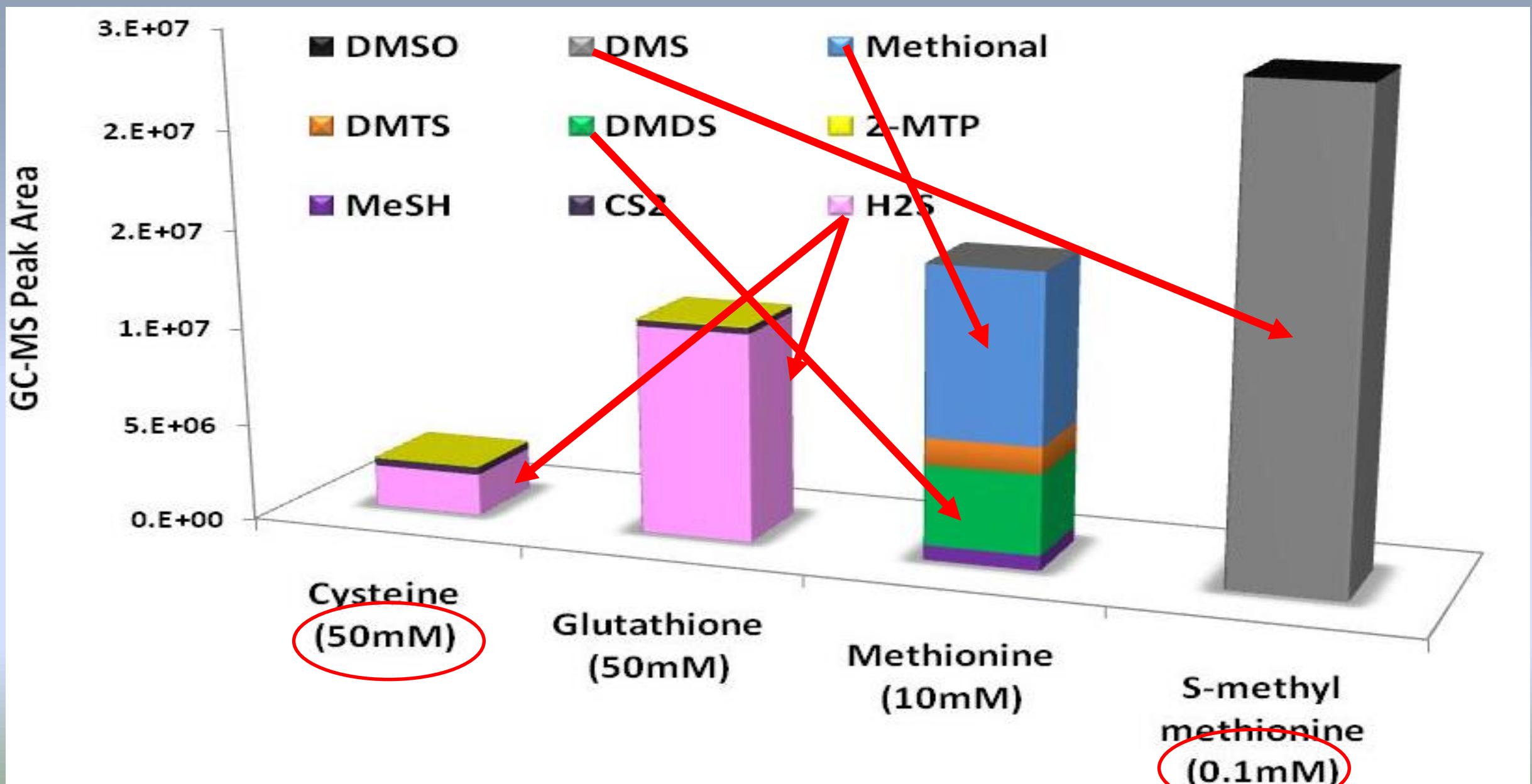


Glutathione



S-Methyl Methionine

S-Amino acids: Thermal breakdown in model GFJ



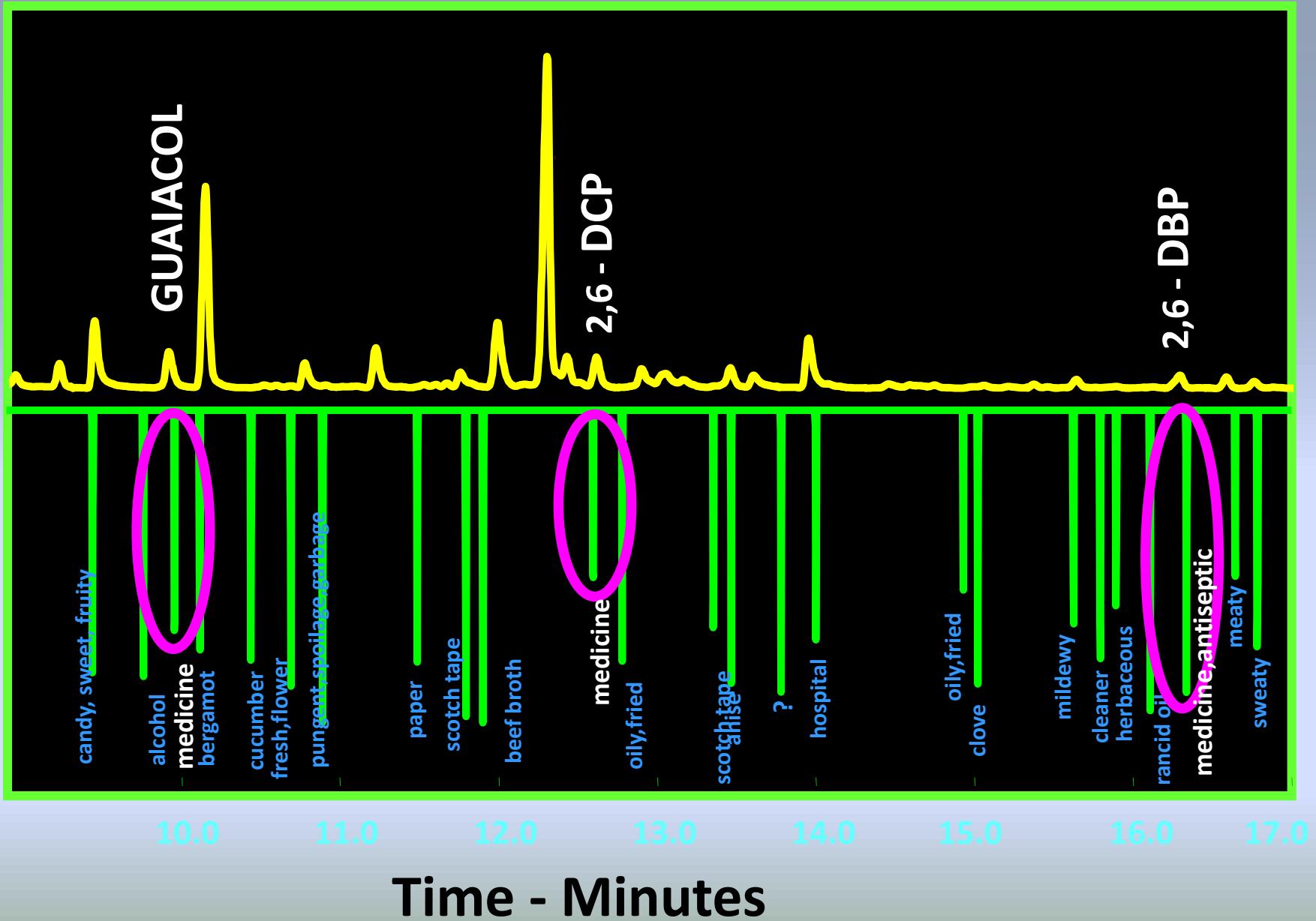
Microbially Induced Off-flavors

Alicyclobacillus acidoterrestris

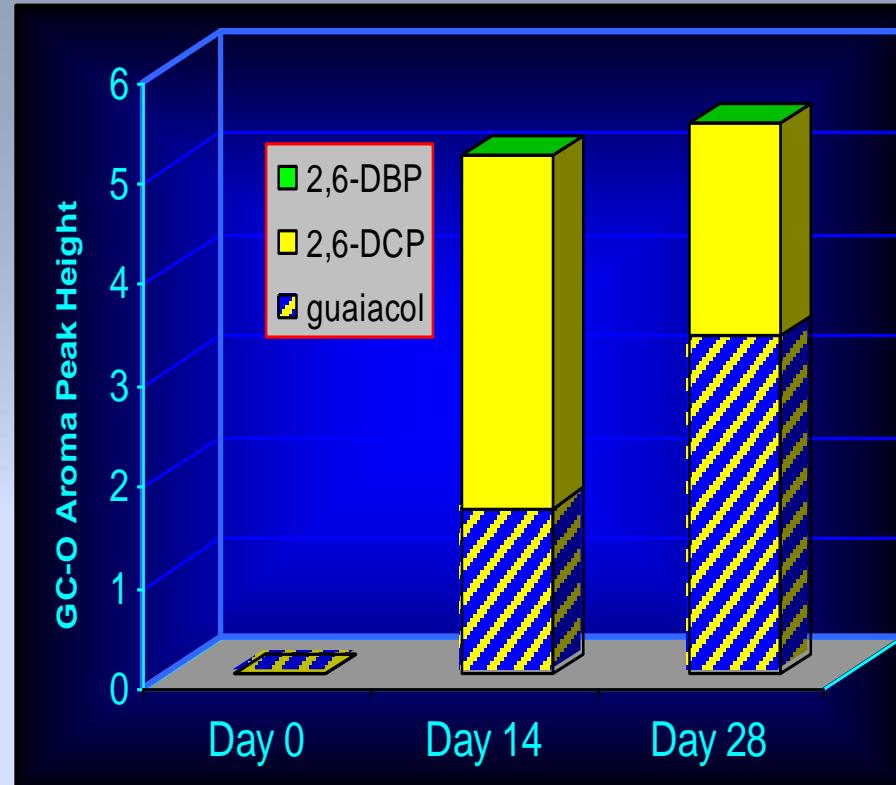
- Non-pathogenic bacteria
- Associated with fruit juice off-flavors
 - flavor defect in apple and orange juices
- Produces flat/sour type spoilage
 - storage temperature critical
 - no gas produced
- Off-aroma
 - medicinal
 - antiseptic
 - disinfectant

A. Cycloheptanicus Day 28

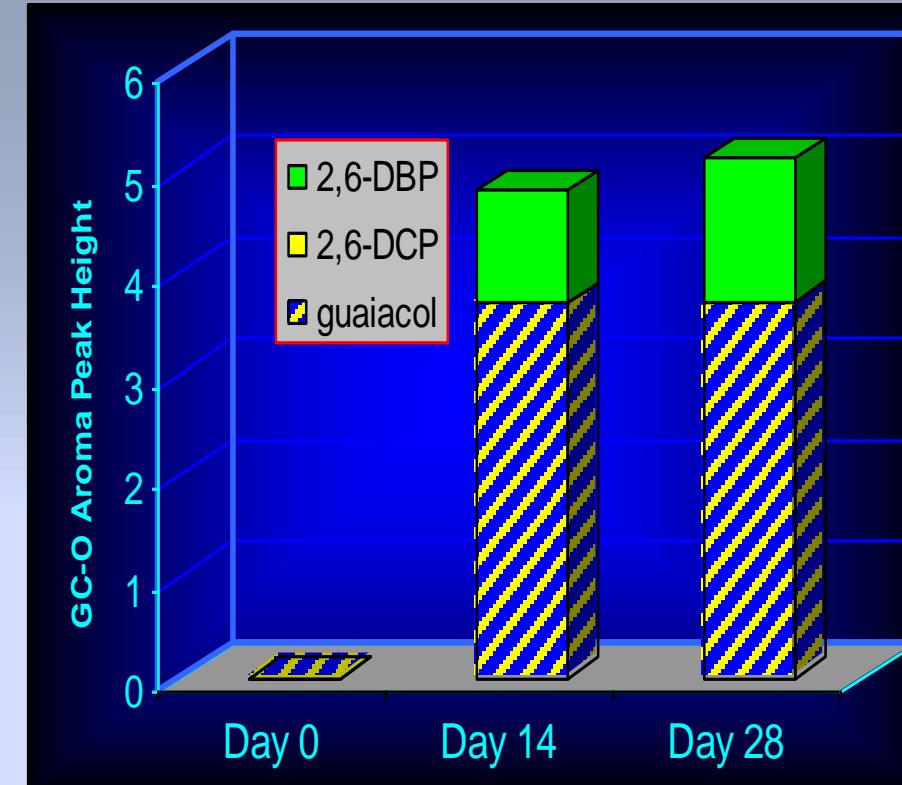
FID Response



A.hesperidum (CECT 5324)



A.acidoterrestis(ATCC 49025)



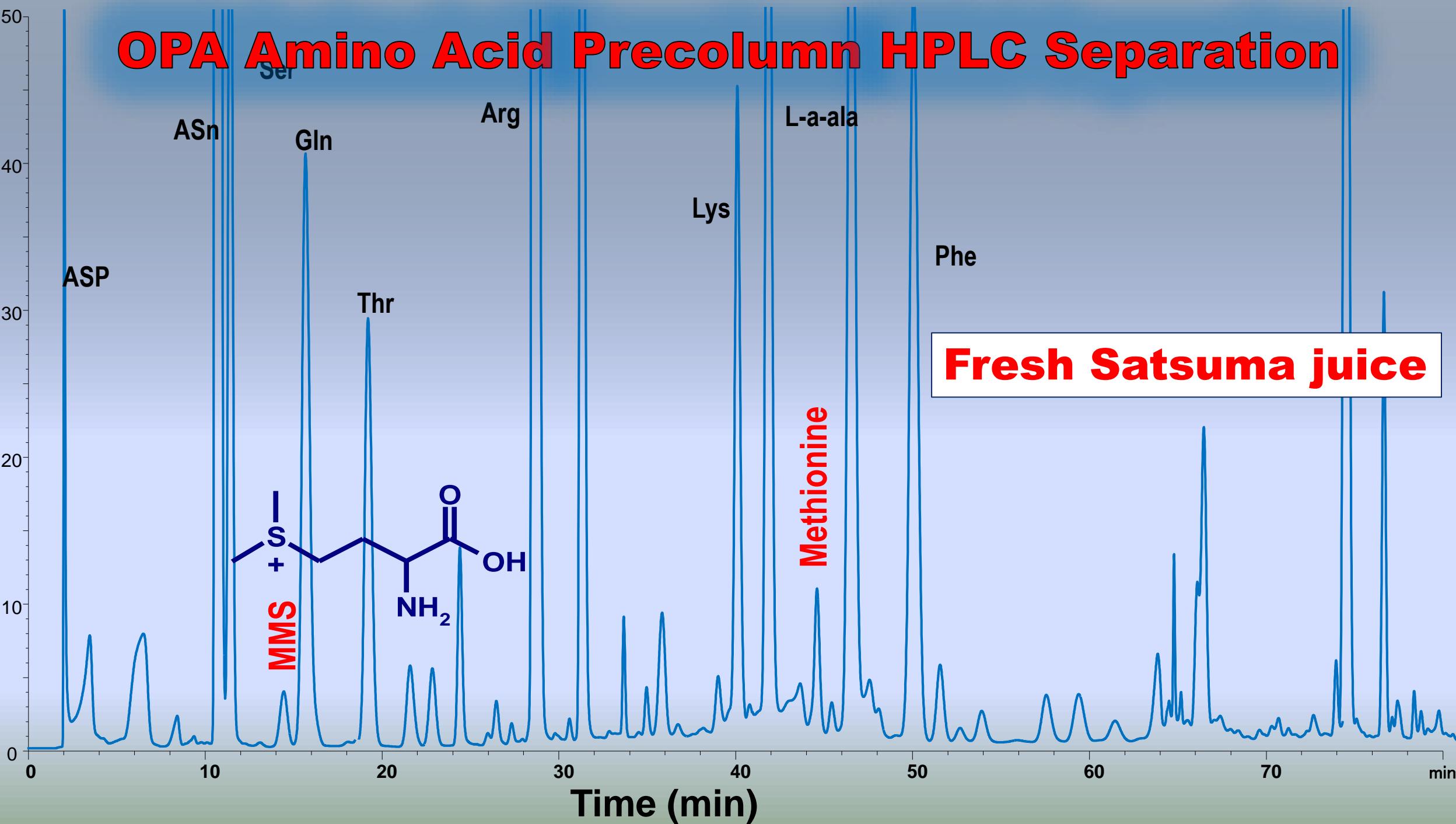
Incubation Time (Days)

Gocmen, D.; Elston, A.; Williams, T.; Parish, M.; Rouseff, R. L., Identification of medicinal off-flavours generated by *Alicyclobacillus* species in orange juice using GC-olfactometry and GC-MS. *Letters Applied Microbiology* **2005**, *40*, 172-77

Perez-Cacho, P. R.; Danyluk, M. D.; Rouseff, R., GC-MS quantification and sensory thresholds of guaiacol in orange juice and its correlation with *Alicyclobacillus* spp. *Food Chemistry* **2011**, *129*, 45-50.

OPA Amino Acid Precolumn HPLC Separation

Fluorescence Intensity



Fresh Satsuma juice



How to Avoid Off-flavor Problems

- **Screen incoming juices for precursors**
- *mandrins have highest MMS*
- *orange and grapefruit lower MMS*
- Sakamoto, K.; Inoue, A.; Nakatani, M.; Kozuka, H.; Ohta, H.; Osajima, Y., S-methylmethionine sulfonium in fruits of citrus hybrids. *Bioscience Biotechnology and Biochemistry* 1996, 60, 1486-1487.
- **Direct “at risk” juices to ref. storage**
- **Pass juice through cation exchange resin**
- *Manabe, T., Dimethyl sulfide as a factor of off-flavor in heat-induced Satsuma mandarin orange (*Citrus unshiu*) juice and its precursor. Hiroshima Nogyo Tanki Daigaku Kenkyu Hokoku 1979, 6, 145-52.*

Questions?

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