

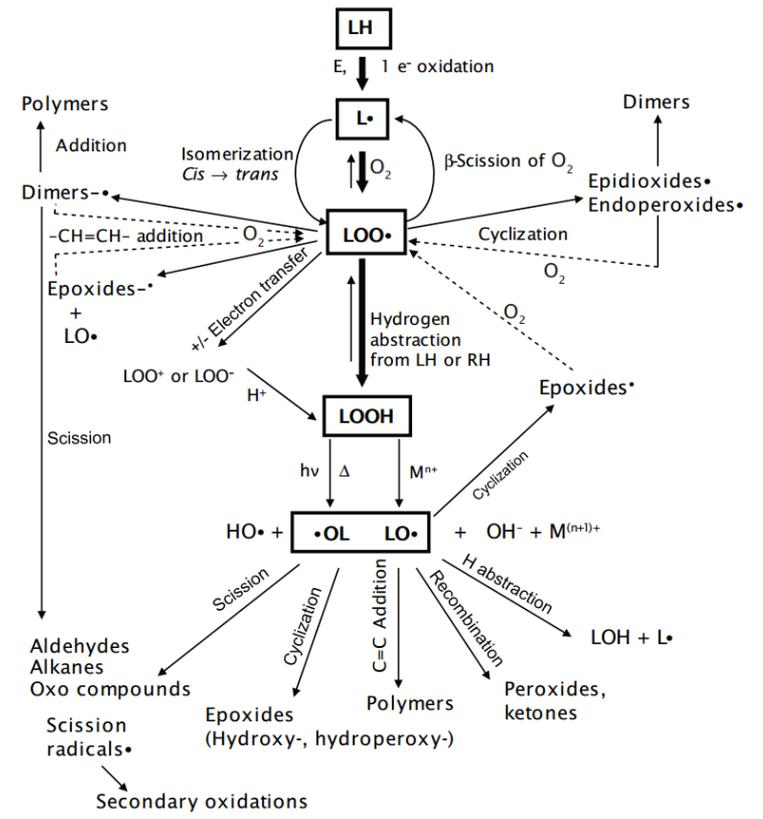
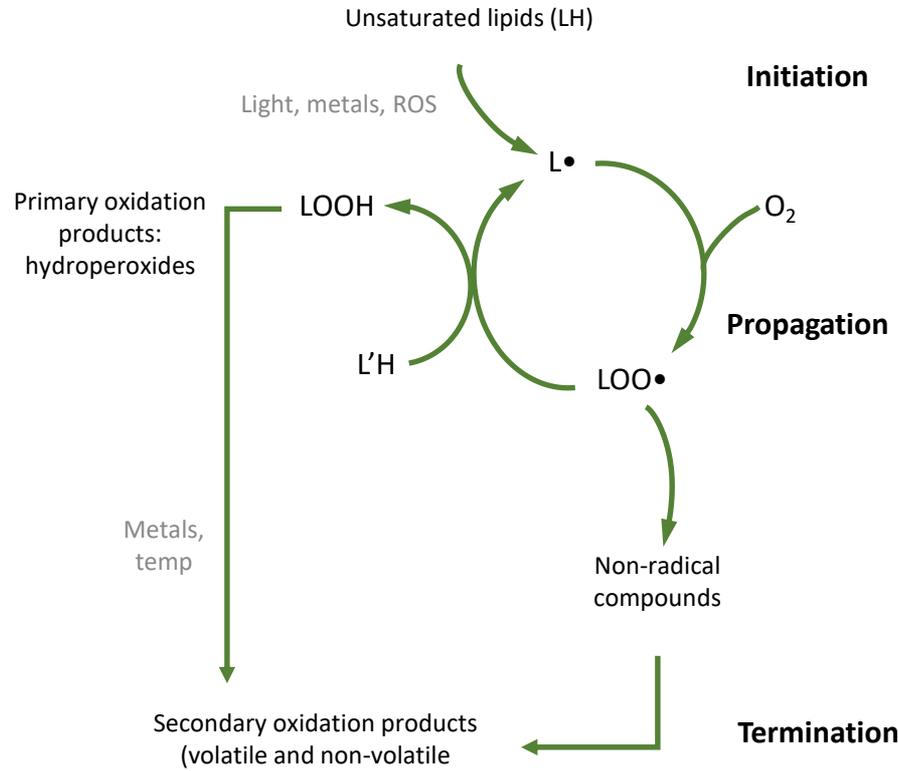
# TruGro<sup>®</sup> AOX

A Technical Approach to Oxidation and Natural Antioxidant Solutions

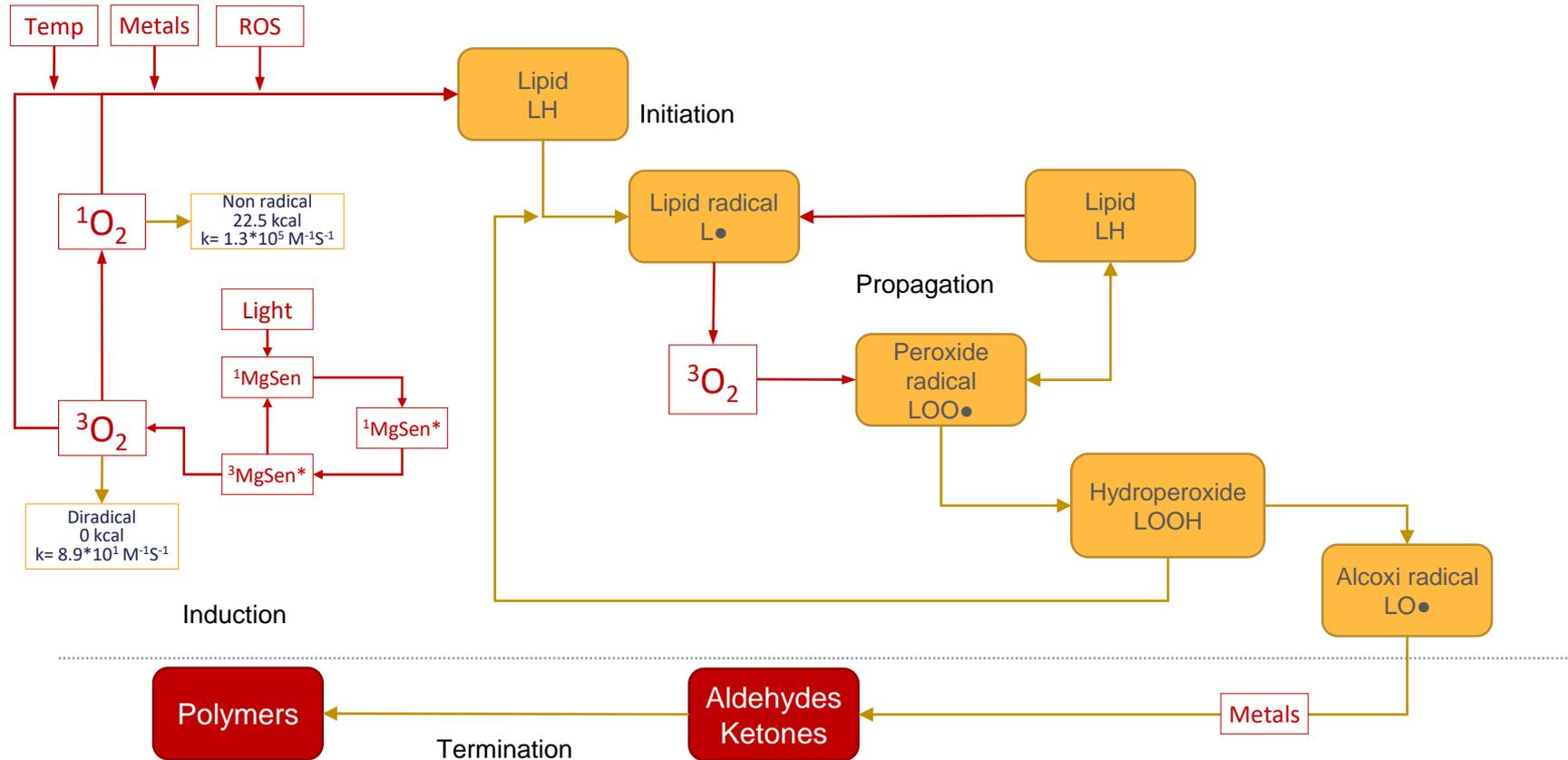


# SCIENCE OF OXIDATION

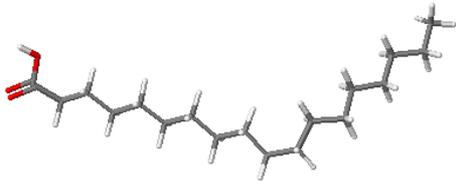
# AUTOOXIDATION PROCESS OF FATS AND OILS



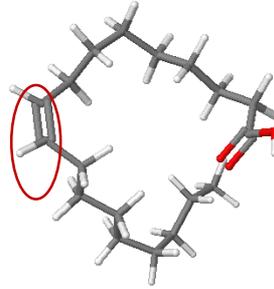
# AUTOOXIDATION PROCESS OF FATS AND OILS



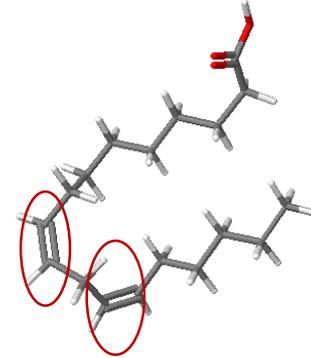
# STRUCTURE OF FATTY ACIDS AND OXIDATIVE STABILITY



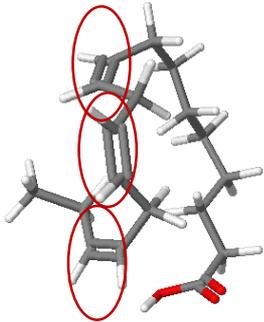
Stearic 18:0



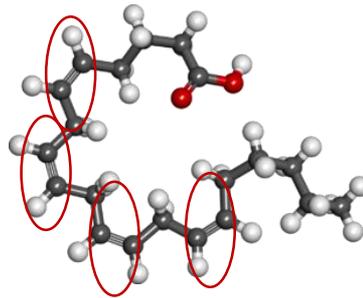
Oleic 18:1



Linoleic 18:2



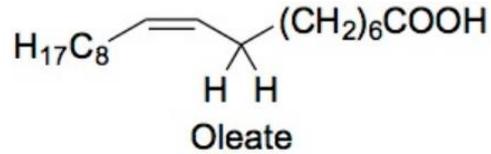
Linolenic 18:3



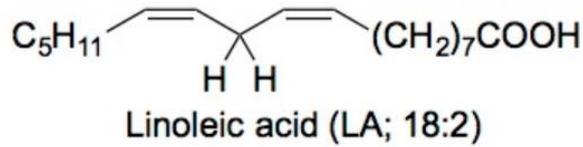
Arachidonic 20:4

| Fatty Acid | Relative Rate of Oxidation |       |
|------------|----------------------------|-------|
|            | Fatty Acid                 | Ester |
| 18:1       | 1                          | 1     |
| 18:2       | 28                         | 41    |
| 18:3       | 77                         | 98    |
| 20:4       |                            | 195   |

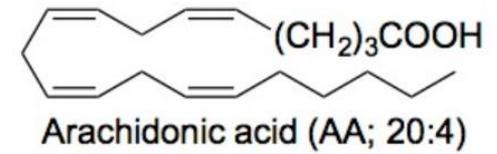
# NUMBER OF DOUBLE BONDS AND SPEED OF AUTOXIDATION:



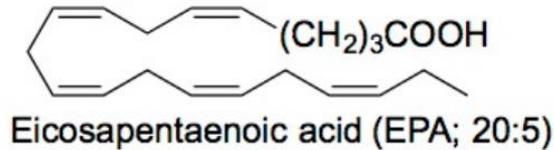
$k=0.8 \text{ M}^{-1}\text{s}^{-1}$



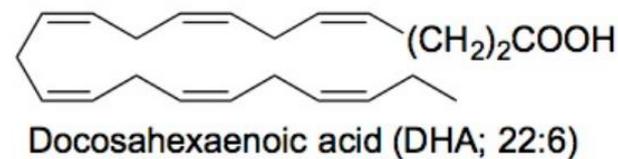
$k=62 \text{ M}^{-1}\text{s}^{-1}$



$k=197 \text{ M}^{-1}\text{s}^{-1}$



$k=249 \text{ M}^{-1}\text{s}^{-1}$

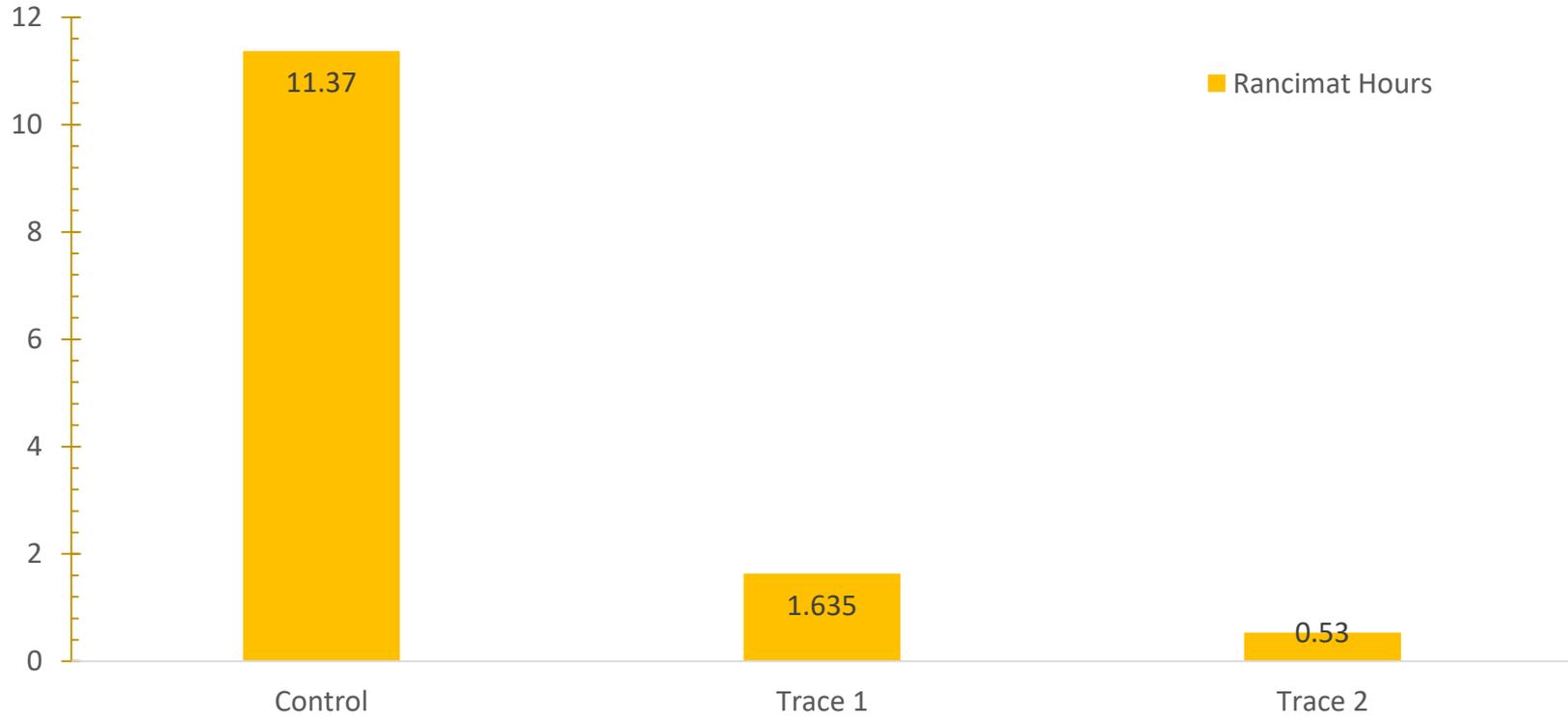


$k=334 \text{ M}^{-1}\text{s}^{-1}$

# FATTY ACIDS PROFILE AND OXIDATIVE POTENTIAL

|               | Saturated | Mono-unsaturated | Poli-unsaturated | MUFA+PUFA | U/S   | Tocopherols Polyphenols | Oxidation Risk |
|---------------|-----------|------------------|------------------|-----------|-------|-------------------------|----------------|
| Coconut       | 86.0      | 5.9              | 1.7              | 7.56      | 0.09  | 0.29                    | *              |
| Bovine tallow | 49.8      | 41.8             | 4.0              | 45.8      | 0.92  | 2.7                     | *              |
| Ovine tallow  | 47.3      | 40.6             | 7.8              | 48.4      | 1.02  | 2.8                     | *              |
| Cotton        | 25.9      | 17.8             | 51.9             | 69.7      | 2.69  | 35.3                    | *              |
| Palm          | 49.3      | 37.0             | 9.3              | 46.3      | 0.94  | 15.9*                   | *              |
| Lard          | 39.2      | 45.1             | 11.2             | 56.3      | 1.44  | 0.6                     | **             |
| Olive         | 13.5      | 73.9             | 10.0             | 83.9      | 6.21  | 15.6*                   | **             |
| Sunflower HO  | 9.0       | 57.3             | 28.9             | 86.2      | 9.58  | 41.1                    | **             |
| Soya          | 14.4      | 23.3             | 57.9             | 81.2      | 5.64  | 9.2                     | ***            |
| Corn          | 12.9      | 27.5             | 54.6             | 82.1      | 6.36  | 14.3                    | ***            |
| Chicken fat   | 29.8      | 44.7             | 20.9             | 65.6      | 2.2   | 2.7                     | ***            |
| Canola        | 7.6       | 62.1             | 25.6             | 87.7      | 11.52 | 48.4                    | ***            |
| Menhaden      | 30.4      | 26.7             | 34.2             | 60.9      | 2.0   |                         | ****           |
| Pilchard      | 29.9      | 33.8             | 31.8             | 65.6      | 2.19  |                         | ****           |
| Yellow grease | 29.9      | 49.5             | 19.4             | 68.9      | 2.3   | 0.6                     | ****           |
| Herring       | 21.3      | 56.5             | 15.6             | 72.1      | 3.38  |                         | ****           |

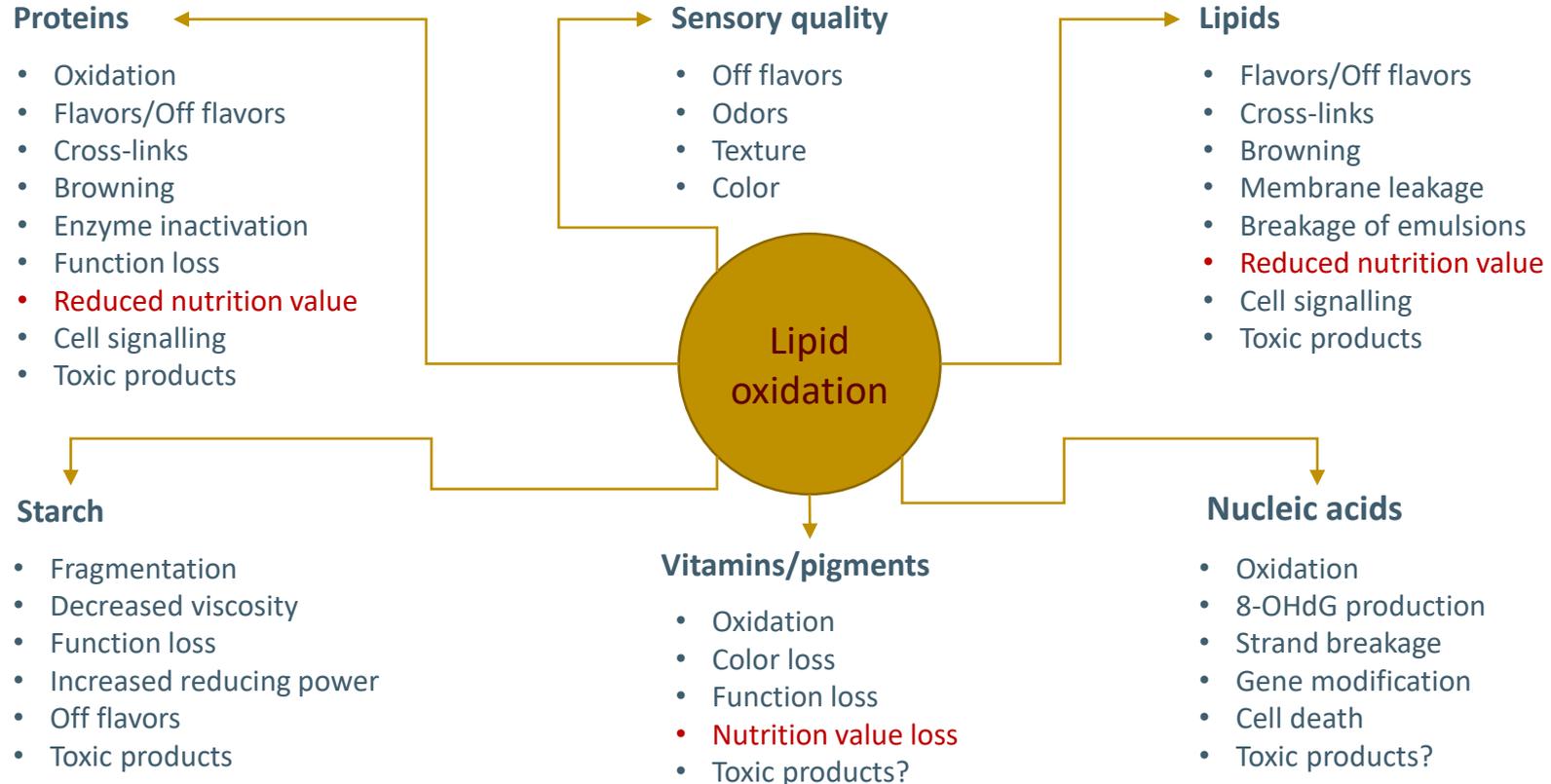
## LIPID STABILITY WITH TRACE MINERALS



# SOME VOLATILES FROM THE DEGRADATION OF FATTY ACIDS

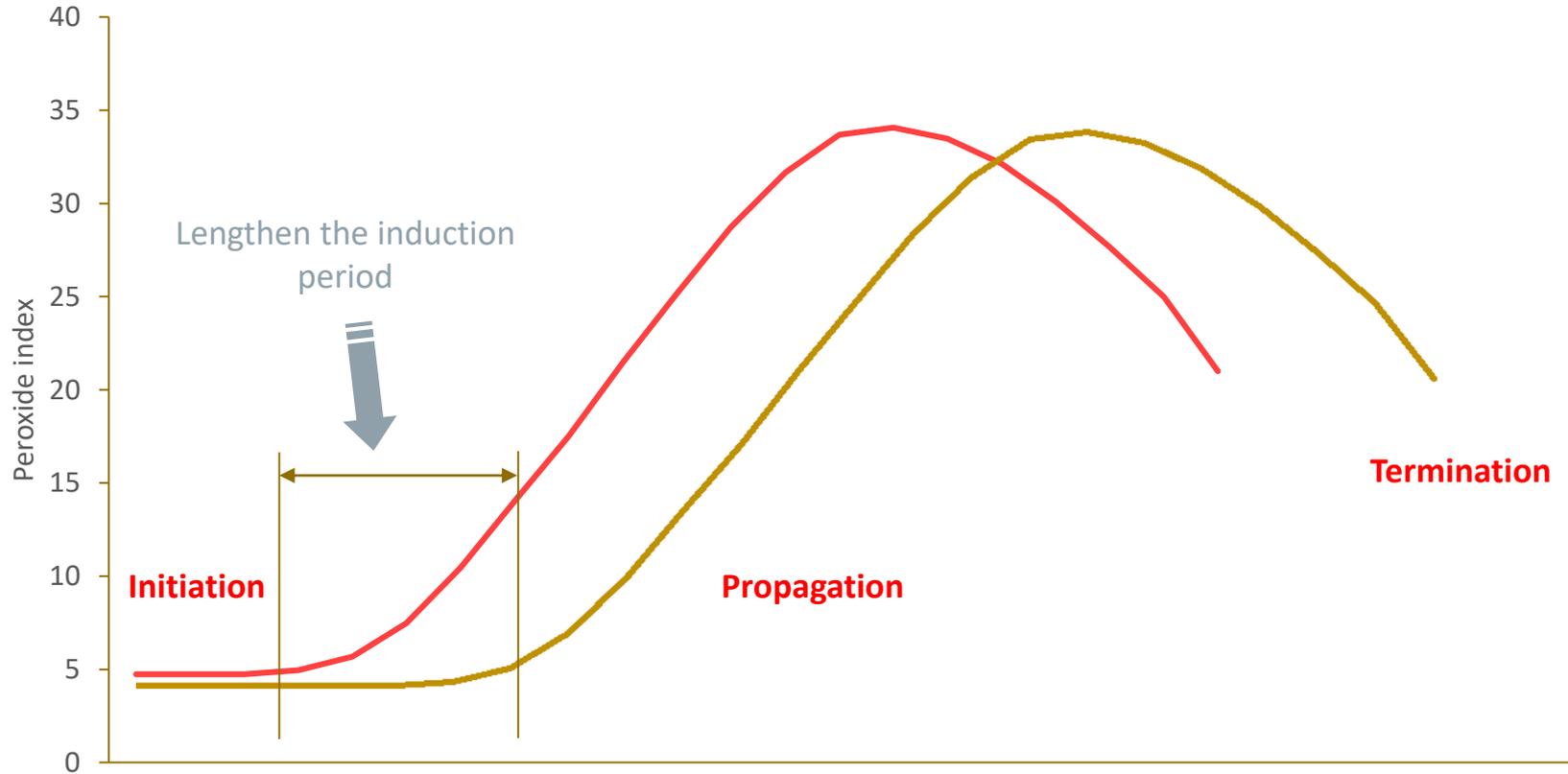
| Compound <sup>3</sup>     | Off-Flavor                  | Threshold Value (ppm) |        |          |
|---------------------------|-----------------------------|-----------------------|--------|----------|
|                           |                             | In Oil                |        | In Water |
|                           |                             | Odor                  | Taste  | Odor     |
| <b>Aldehydes</b>          |                             |                       |        |          |
| Pentanal                  | Sharp, bitter almond        | 0.24                  | 0.15   | 0.012    |
| Hexanal                   | Green—fruity, bitter almond | 0.32                  | 0.08   | 0.008    |
| Octanal                   | Fatty, soapy—fruity         | 0.32                  | 0.04   | 0.0007   |
| Nonanal                   | Tallowy, soapy—fruity       | 13.5                  | 0.2    | 0.001    |
| Decanal                   | Orange peels                | 6.7                   | 0.7    | 0.0001   |
| Nonenal (3c)              | Green cucumber              | 0.25                  | 0.03   |          |
| Nonenal (2t)              | Tallowy, starch—glue        | 3.5                   | 0.04   | 0.0008   |
| Nonadienal (2t,4t)        | Fatty, oily                 | 2.5                   | 0.46   |          |
| Nonadienal (2t,6c)        | Cucumbers                   | 0.01                  | 0.0015 |          |
| Nonadienal (2t,6t)        | Tallowy, green              | 0.21                  | 0.018  |          |
| Decadienal (2t,4c)        | Frying odor                 |                       | 0.02   |          |
| Decadienal (2t,4t)        | Deep-fried                  | 2.15                  | 0.1    |          |
| <b>Ketones and furans</b> |                             |                       |        |          |
| 1-Pentene-3-one           | Sharp, fishy                |                       | 0.003  |          |
| 1 -Octen-3-one            | Metallic                    |                       |        |          |
| 1 -Octen-3-ol             | Moldy, mushroomy            | 0.077                 | 0.0001 | 0.00009  |
| 2-Pentylfuran             | Buttery, beany              | 2                     |        |          |
| 2-(1-Pentenyl) furan      | Licorice                    | 2 to 6                |        |          |

# EFFECTS OF OXIDATION IN FOOD AND FEED INGREDIENTS



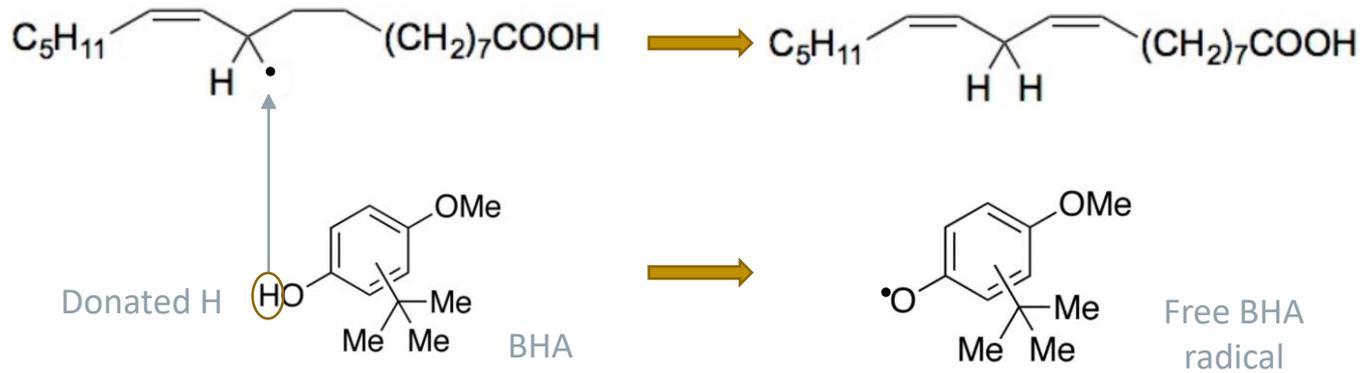
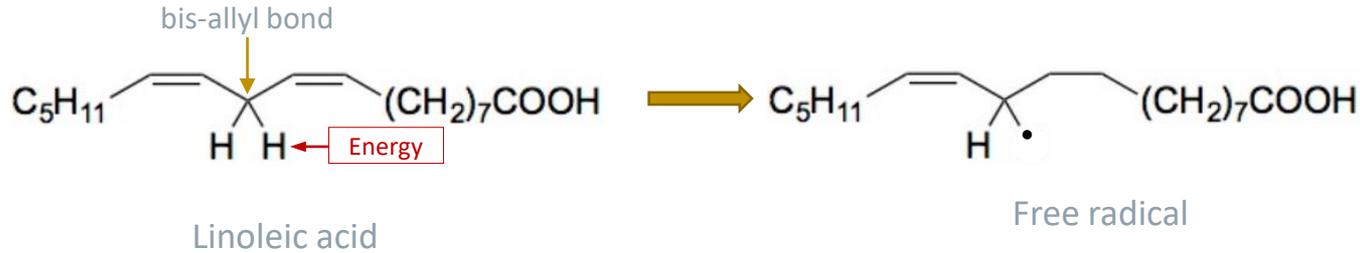
# TECHNOLOGY OF ANTIOXIDANTS

# THE ROLE OF ANTIOXIDANTS



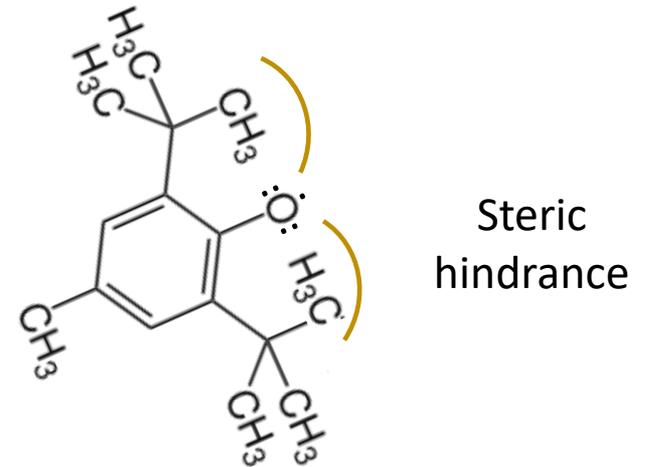
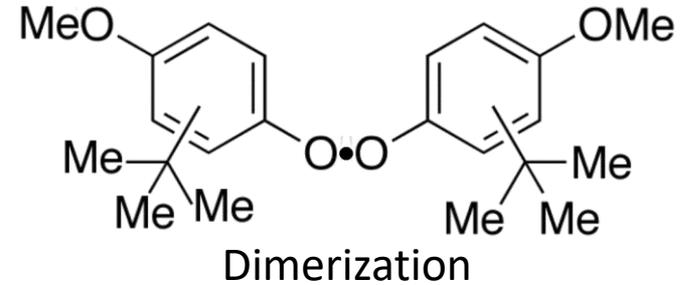
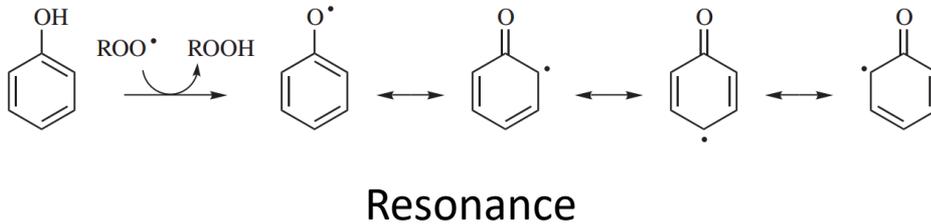
# RADICALARY REACTIONS AND ANTIOXIDANT MECHANISM

C=C-C=C: 75 kcal/mol  
 C=C: 88 kcal/mol  
 C-C: 101 kcal/mol



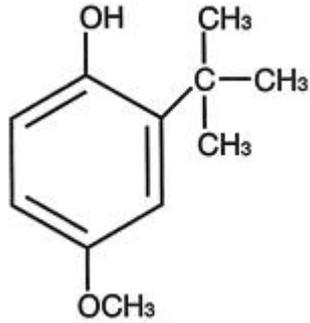
## • Neutralization systems

- Dimerization: two radicals together block the active site.
- Resonance: delocalization of electron in phenol molecules
- Steric hindrance: physical occupation by tert-butyl groups

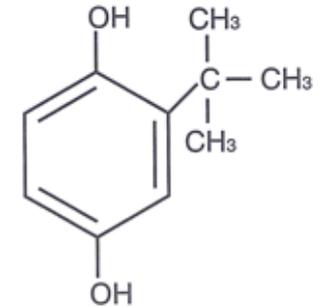
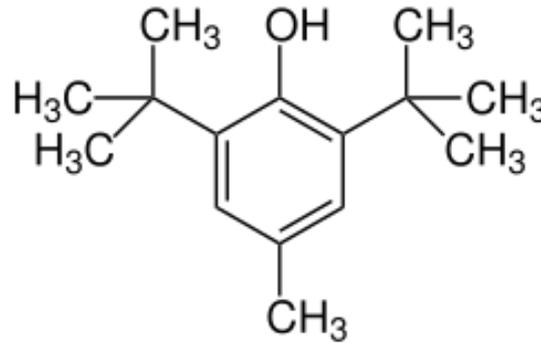


# TYPES OF ANTIOXIDANTS: SYNTHETIC

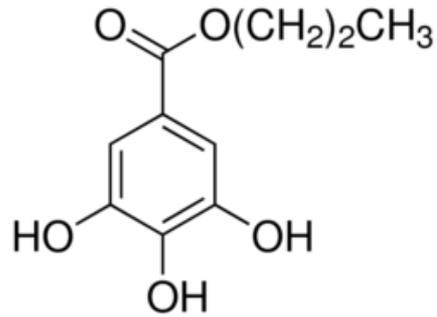
BHA



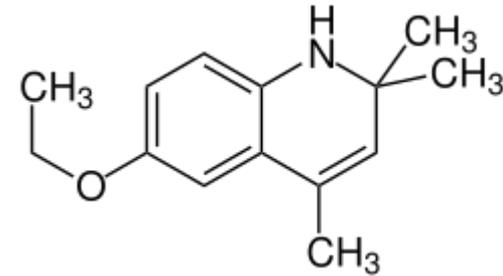
BHT



TBHQ

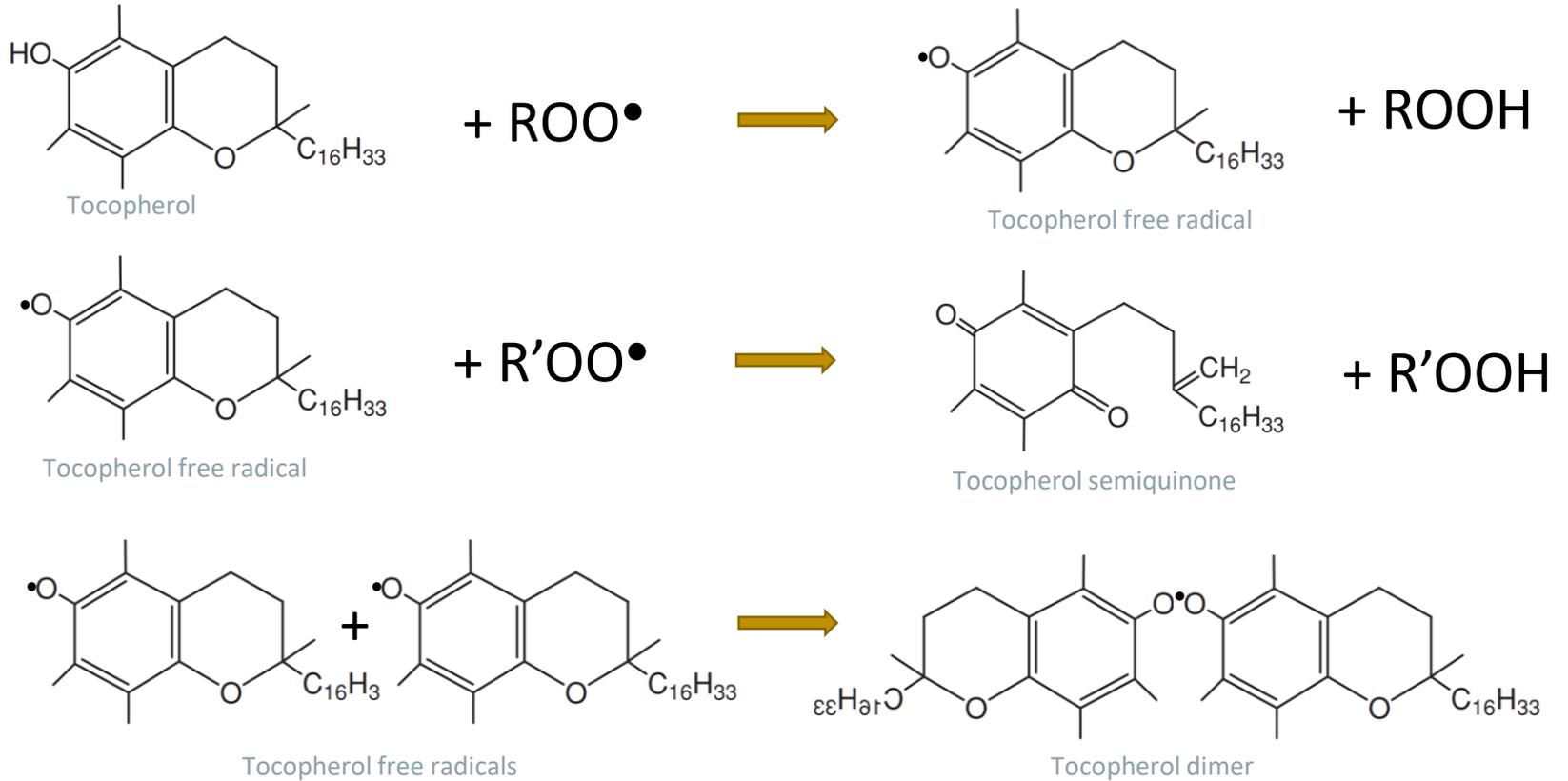


Propyl gallate

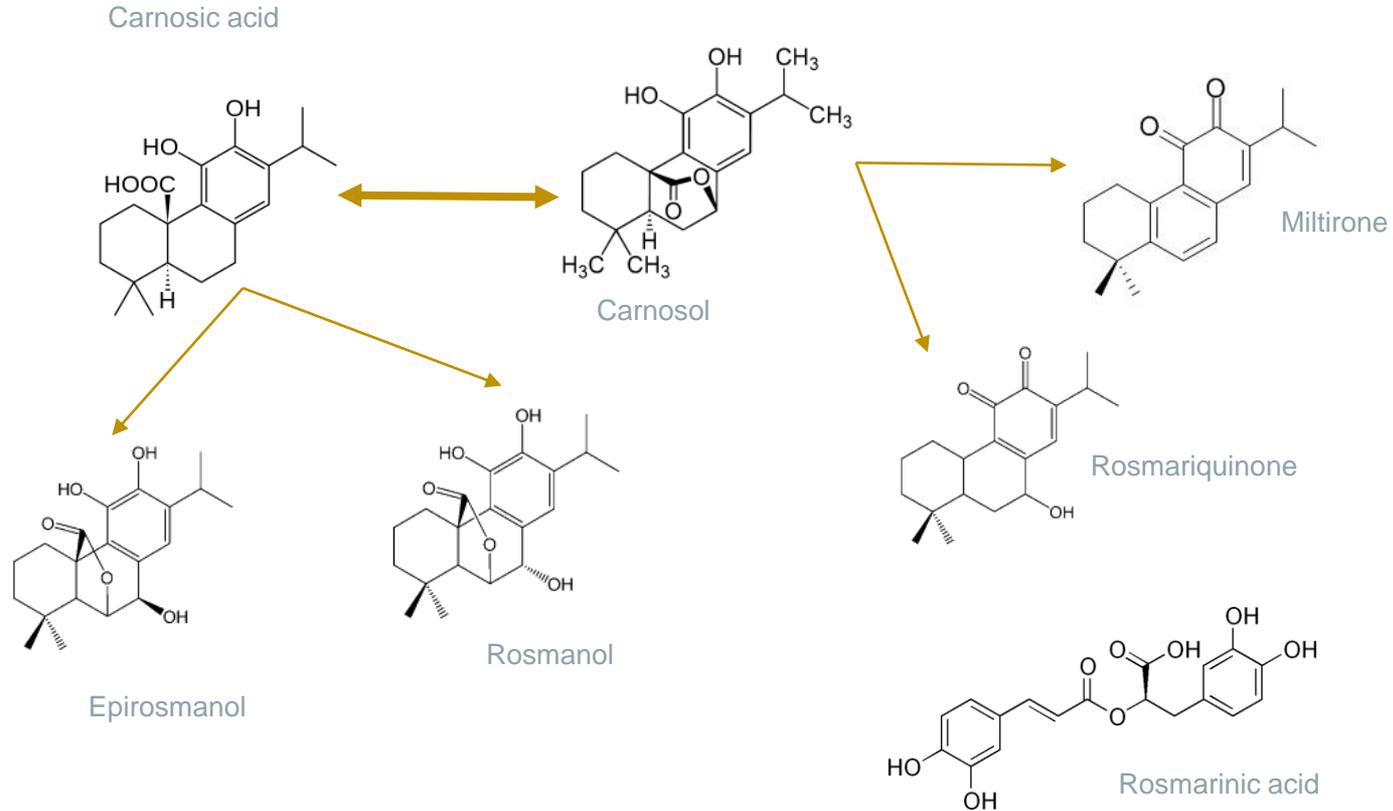


ETQ

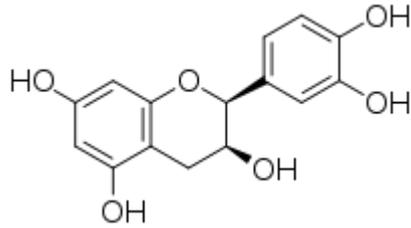
# NATURAL ANTIOXIDANTS: TOCOPHEROLS



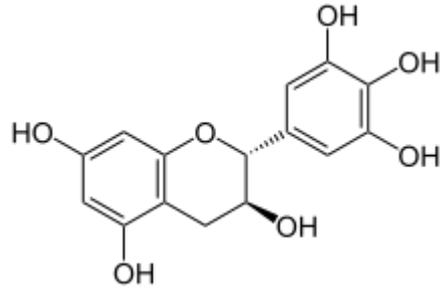
# NATURAL ANTIOXIDANTS: DITERPENES FROM ROSEMARY



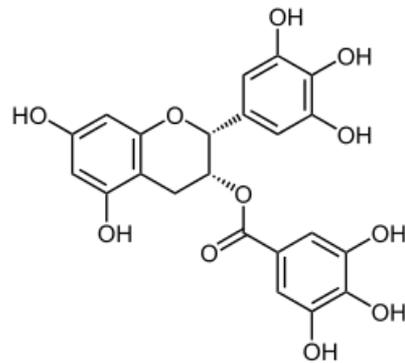
# NATURAL ANTIOXIDANTS: CATECHIN AND GALLIC ACID DERIVATIVES



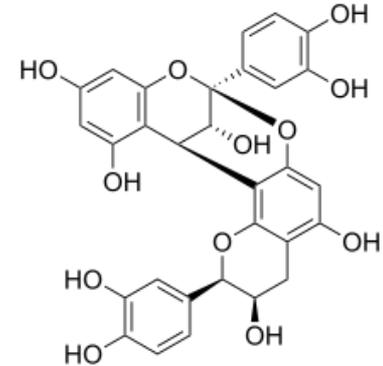
Catechin



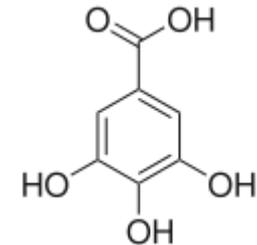
Galocatechol



Epigallocatechin gallate

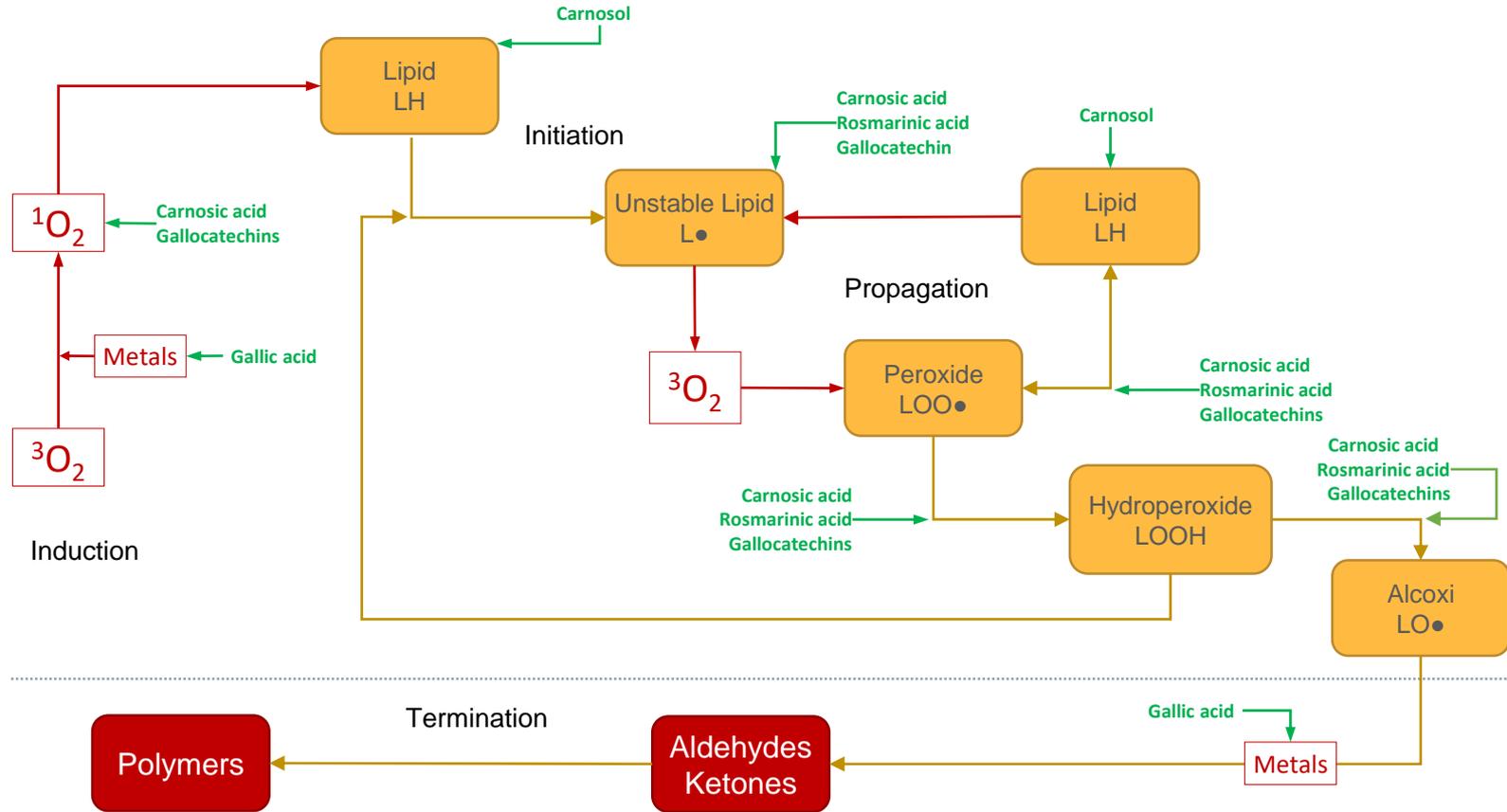


Procyanidin A2

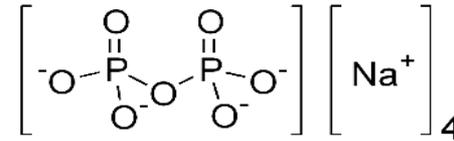


Gallic acid

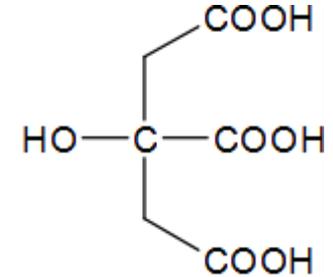
# ANTIOXIDANT FUNCTIONS OF POLYPHENOLS IN LIPID OXIDATION



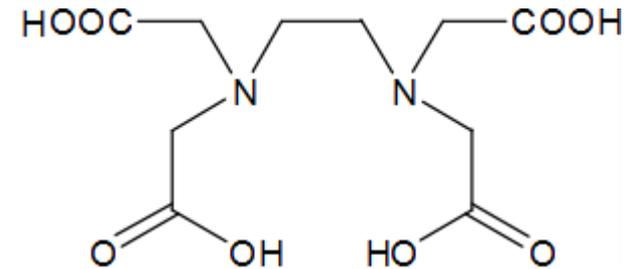
- Block metals and avoid their activity upon the antioxidant
- Reversible: release metal ions in the gut
  - Citric acid
  - Ascorbic acid
  - Tartaric acid
  - Phosphates
- Irreversible: do not release metal ions
  - EDTA



Trisodium phosphate

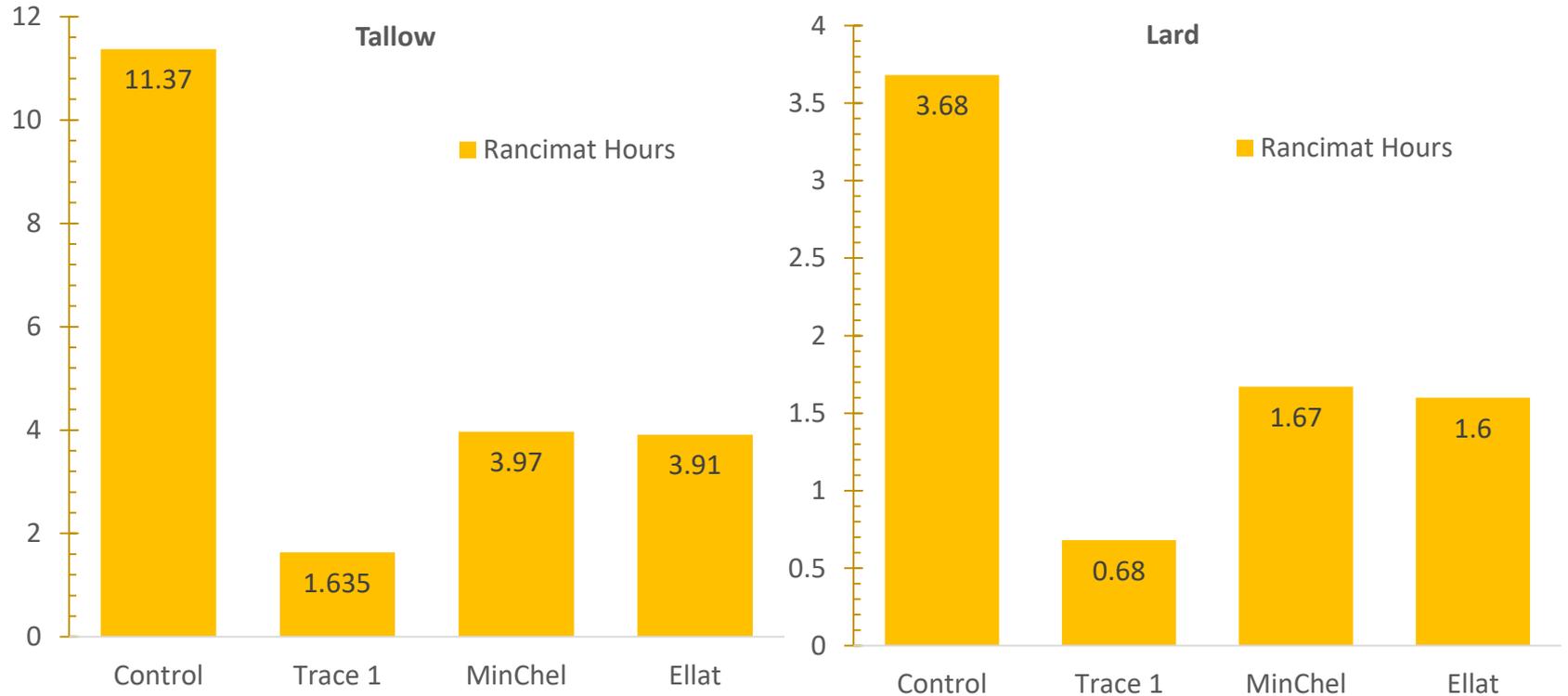


Citric acid



EDTA

# TRACE MINERALS AND CHELATING AGENTS ON LIPID STABILITY

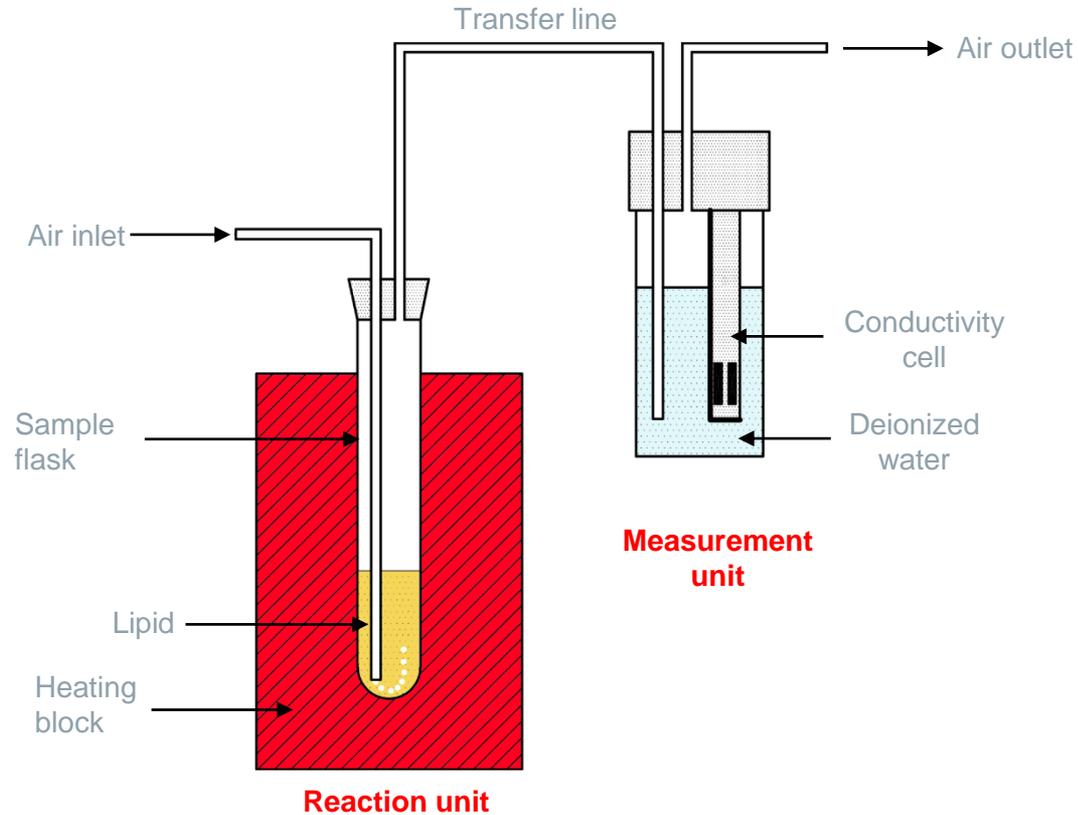


Trace 1: 1.8 ppm Fe + 1.5 ppm Cu. Minchel: citric acid + sodium phosphate, 64 ppm. Ellat: ellagitannins, 60 ppm. Layn, 2019

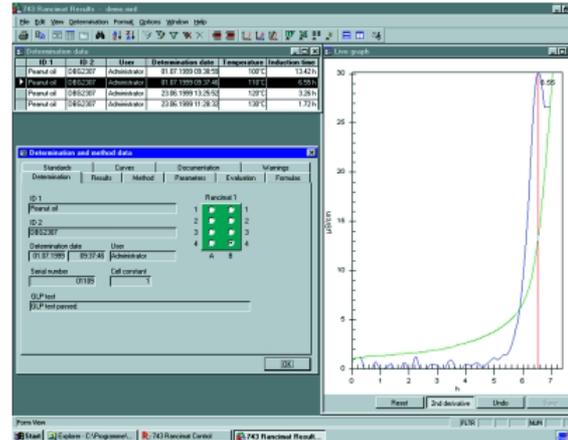
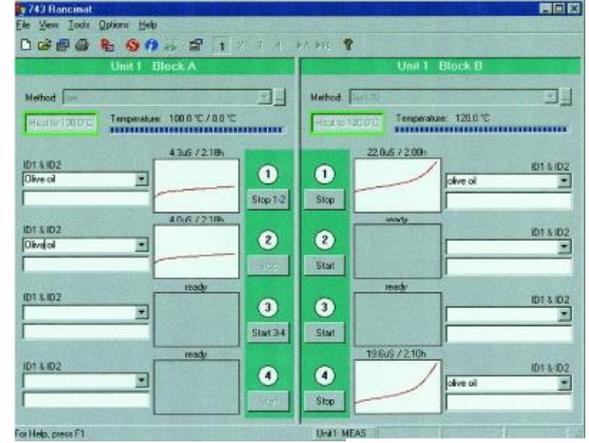
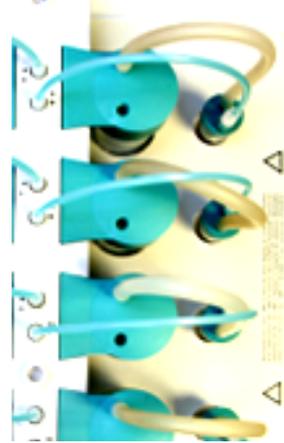
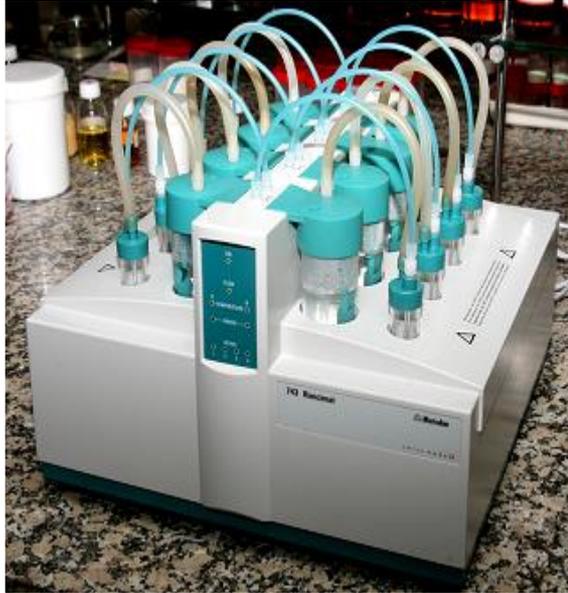
# TruGro<sup>®</sup> AOX ASSESSMENT

- Indicator: Oil Stability Index (OSI)
- Technology: Rancimat
- Substrate: several types of lipids
- Reference: synthetic antioxidants
  - BHA 150 ppm
  - BHT 150 ppm

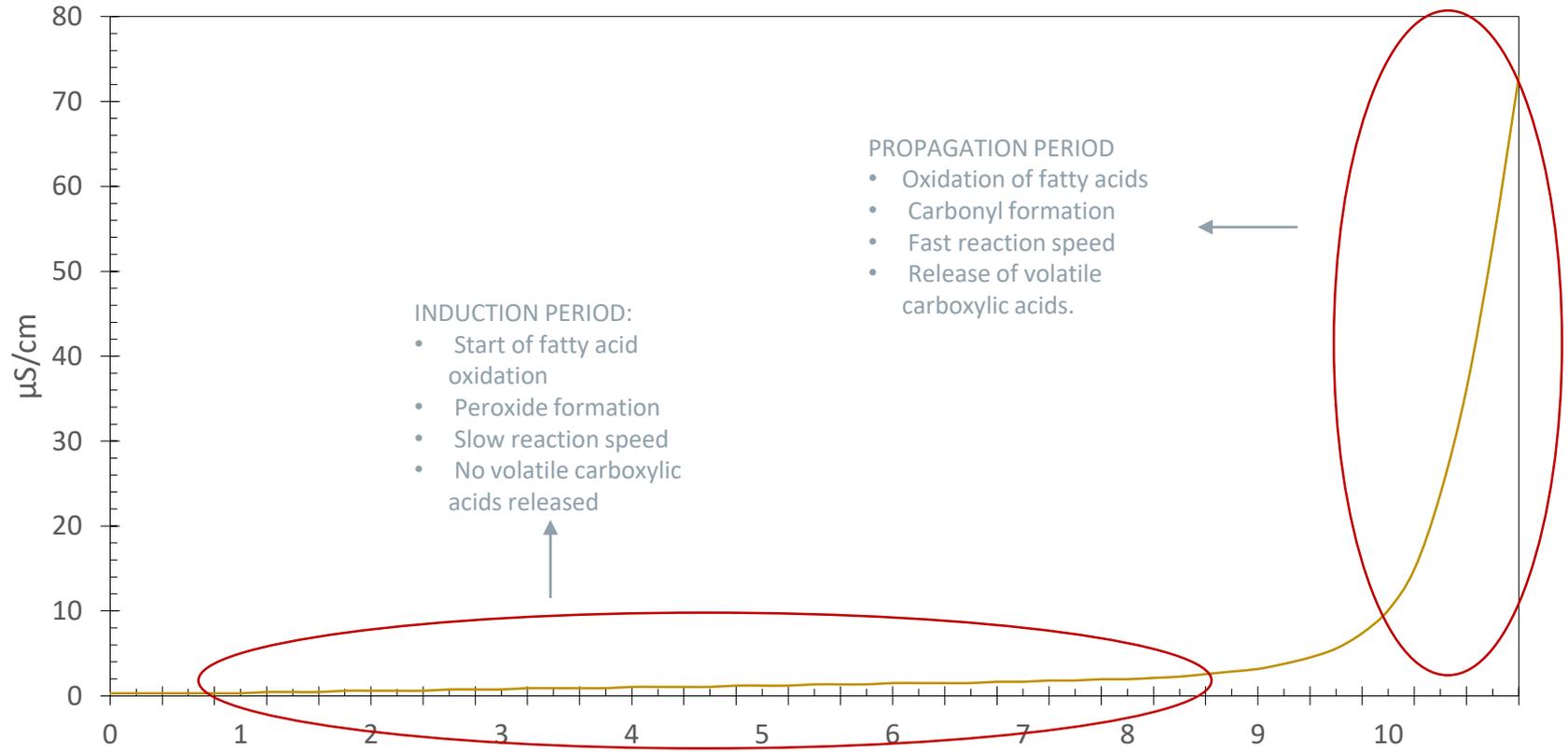
## RANCIMAT BLOCK DIAGRAM



# RANCIMAT DEVICE



# RESULTS PLOT



# SUBSTRATE CHARACTERISTICS

| Fatty acid, %              | Sunflower    | Soy         | Palmolein   | Lard        | Tallow      |
|----------------------------|--------------|-------------|-------------|-------------|-------------|
| Caprilic                   | 0.04         |             | 0.05        |             |             |
| Capric                     | 0.03         |             | 0.04        |             |             |
| Lauric                     | 0.22         | 0.01        | 0.42        | 0.11        | 0.09        |
| Myristic                   | 0.13         | 0.08        | 0.83        | 1.4         | 3.24        |
| Myristoleic                |              | 0.01        | 0.0         | 0.02        | 0.64        |
| Pentadecanoic              | 0.02         | 0.13        | 0.05        | 0.07        | 0.62        |
| Palmitic                   | 3.87         | 10.58       | 37.2        | 25.23       | 25.5        |
| Palmitoleic                | 0.12         | 0.12        | 0.2         | 1.79        | 3.46        |
| Margaric                   | 0.03         | 0.09        | 0.09        | 0.41        | 1.52        |
| Margaroleic                | 0.04         | 0.05        | 0.03        | 0.41        | 0.59        |
| Stearic                    | 3.4          | 3.53        | 4.98        | 17.62       | 17.79       |
| Oleic                      | 78.35        | 26.52       | 43.88       | 35.63       | 36.29       |
| Trans C18:1 isomers        | 0.02         | 0.04        | 0.11        | 0.25        | 5.08        |
| Linoleic                   | 11.51        | 51.18       | 10.83       | 14.08       | 2.92        |
| Trans C18:2 isomers        | 0.16         | 0.19        | 0.26        | 0.07        | 0.57        |
| Linolenic                  | 0.01         | 5.67        | 0.05        | 0.084       | 0.28        |
| Saturated fatty acids, %   | 7.74         | 14.42       | 43.66       | 44.84       | 48.76       |
| Unsaturated fatty acids, % | 90.21        | 83.78       | 55.36       | 52.33       | 49.83       |
| <b>U/S ratio</b>           | <b>11.66</b> | <b>5.81</b> | <b>1.27</b> | <b>1.17</b> | <b>1.02</b> |

# RESULTS FROM RANCIMAT DETERMINATIONS

| Polyphenol-based antioxidants against industry-standard synthetics |       |       |       |                      |
|--|-------|-------|-------|----------------------|
|  | Run 1 | Run 2 | Mean  | % stability increase |
| <b>Soy oil</b>   |       |       |       |                      |
| Blank  | 5.65  | 5.60  | 5.63  |                      |
| BHA 150 ppm  | 5.74  | 5.62  | 5.68  | 0.89                 |
| BHT 150 ppm  | 6.24  | 6.05  | 6.15  | 9.24                 |
| AOX 1112 150 ppm   | 6.68  | 6.94  | 6.81  | 20.96                |
| AOX 1113 150 ppm   | 6.32  | 6.53  | 6.43  | 14.21                |
| <b>Tallow</b>  |       |       |       |                      |
| Blank  | 18.56 | 17.60 | 18.08 |                      |
| BHA 150 ppm  | 36.78 | 36.44 | 36.61 | 102.49               |
| BHT 150 ppm  | 21.35 | 21.41 | 21.38 | 18.25                |
| AOX 1112 150 ppm   | 45.12 | 43.22 | 44.17 | 144.30               |
| AOX 1113 150 ppm   | 47.90 | 48.52 | 48.21 | 166.65               |
| <b>HO Sunflower</b>  |       |       |       |                      |
| Blank  | 15.04 | 14.56 | 14.80 |                      |
| BHA 150 ppm  | 16.62 | 16.55 | 16.58 | 12.03                |
| BHT 150 ppm  | 17.32 | 17.27 | 17.29 | 16.82                |
| AOX 1112 150 ppm   | 20.96 | 21.18 | 21.07 | 42.36                |
| AOX 1113 150 ppm   | 19.09 | 19.19 | 19.14 | 29.32                |

# COMPARISON WITH NATURAL ANTIOXIDANTS IN UNSATURATED AND SATURATED LIPIDS

| Effect of several antioxidants on sunflower oil stability |       |       |      |                      |
|---|-------|-------|------|----------------------|
|   | Run 1 | Run 2 | AVG  | % stability increase |
| Blank   | 2.61  | 2.54  | 2.58 |                      |
| Rosemary extract 1, 650 ppm                               | 2.88  | 2.93  | 2.91 | 12.82                |
| Rosemary extract 2, 650 ppm                               | 2.82  | 2.79  | 2.81 | 8.93                 |
| Rosemary extract 3, 650 ppm                               | 2.94  | 2.99  | 2.97 | 15.15                |
| Layn Green Tea extract OS, 1000 ppm                       | 5.09  | 5.08  | 5.09 | 97.48                |

| Effect of several antioxidants on bovine tallow stability |       |       |       |                      |
|---|-------|-------|-------|----------------------|
|   | Run 1 | Run 2 | AVG   | % stability increase |
| Blank   | 9.90  | 9.87  | 9.88  |                      |
| Rosemary extract 1, 650 ppm                               | 29.6  | 29.2  | 29.40 | 397.4                |
| Rosemary extract 2, 650 ppm                               | 30.51 | 30.11 | 30.31 | 406.6                |
| Rosemary extract 3, 650 ppm                               | 30.62 | 30.30 | 30.46 | 408.1                |
| Layn Green Tea extract OS, 1000 ppm                       | 44.23 | 44.74 | 44.48 | 550.0                |

# CONCLUSION

# ATTRIBUTES OF POLYPHENOL-BASED LIPID ANTI OXIDANTS

- **Adequacy**
  - By using a selected combination of standardized botanical extracts with high polyphenol content, it has been possible to increase lipid stability under the analytical conditions used.
  - This research shows selected polyphenol combinations improves on the antioxidant protection afforded by two synthetic compounds.
- **Specificity**
  - This research shows the lipid's unsaturation index influences the efficacy of polyphenol combinations. In practical terms, this means the knowledge of the U/S ratio would modulate usage for a particular application, mandating different ingredient combinations for maximum response.
- **Implications**
  - When consumer choice forces the level of synthetics being reduced or altogether removed, polyphenol antioxidants constitute an alternative solution to lipid stability in pet food systems.

- **TruGro<sup>®</sup> AOX 1112**

- A selected combination of standardized botanical extracts being the choice antioxidant product for lipids with high unsaturation index: soy oil, canola oil, peanut oil, sunflower oil or palmoleins.
- Add to dry feed at a rate of 30 ppm/1% fat, for 18 months shelf life

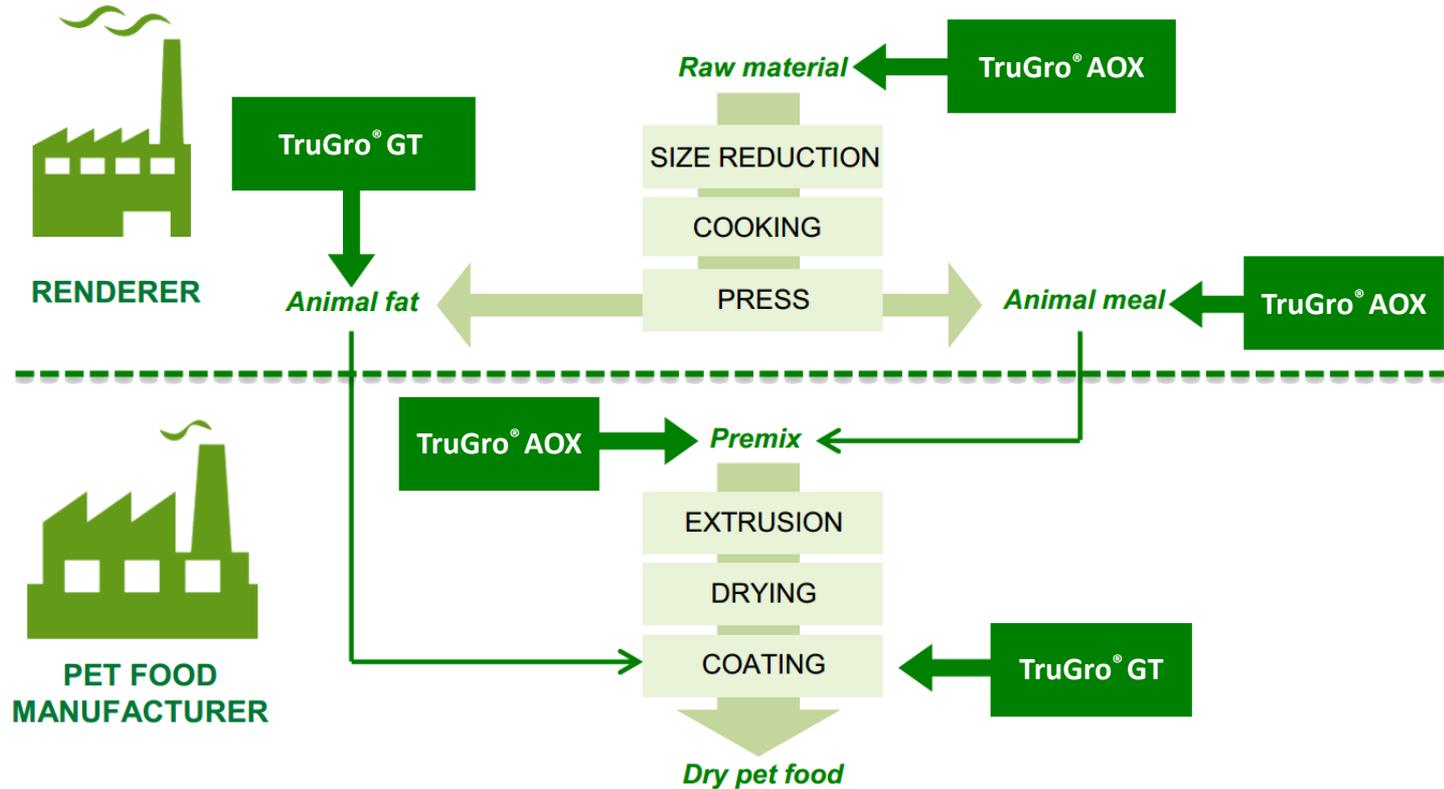
- **TruGro<sup>®</sup> AOX 1113**

- A selected combination of standardized botanical extracts being the choice antioxidant product for lipids with low unsaturation index: lard or tallow
- Add to dry feed at a rate of 30 ppm/1% fat, for 18 months shelf life

- **TruGro<sup>®</sup> GT OS**

- A selected combination of standardized botanical extracts being the choice antioxidant product for lipids with high unsaturation index for external kibble coating: soy oil, canola oil, peanut oil, sunflower oil, or chicken oil.
- Add to cover lipids at a rate of 10 ppm/1% fat, for extended shelf life.

# ANTIOXIDANT APPLICATION PATTERN IN THE INDUSTRY



# THANK YOU

Questions?

Enquiries?

Sample requests?