# Antioxidants from Cereal Grain and Their Byproduct Proteins

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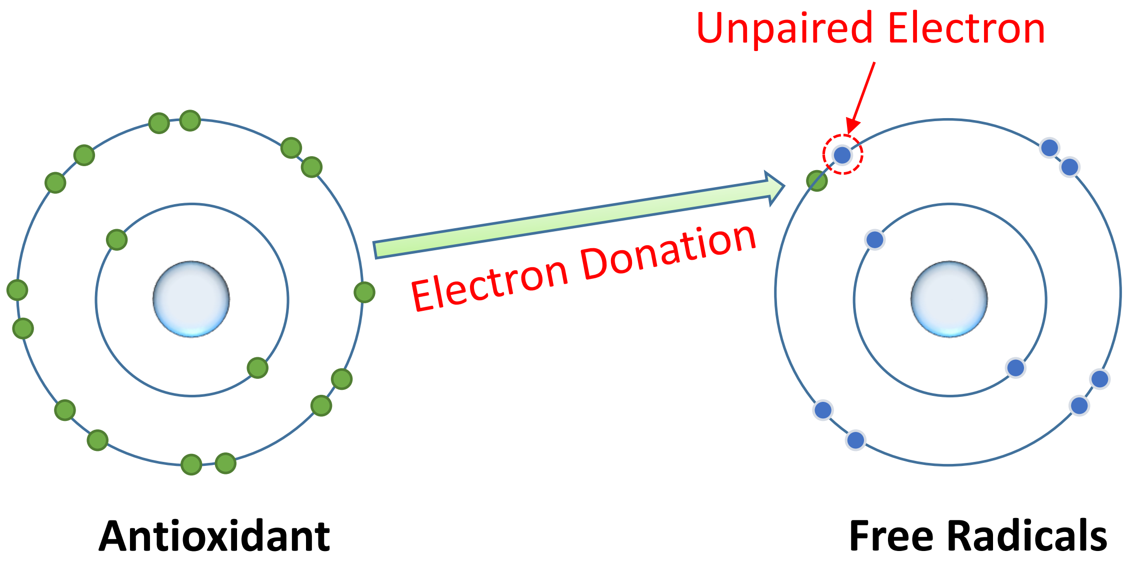
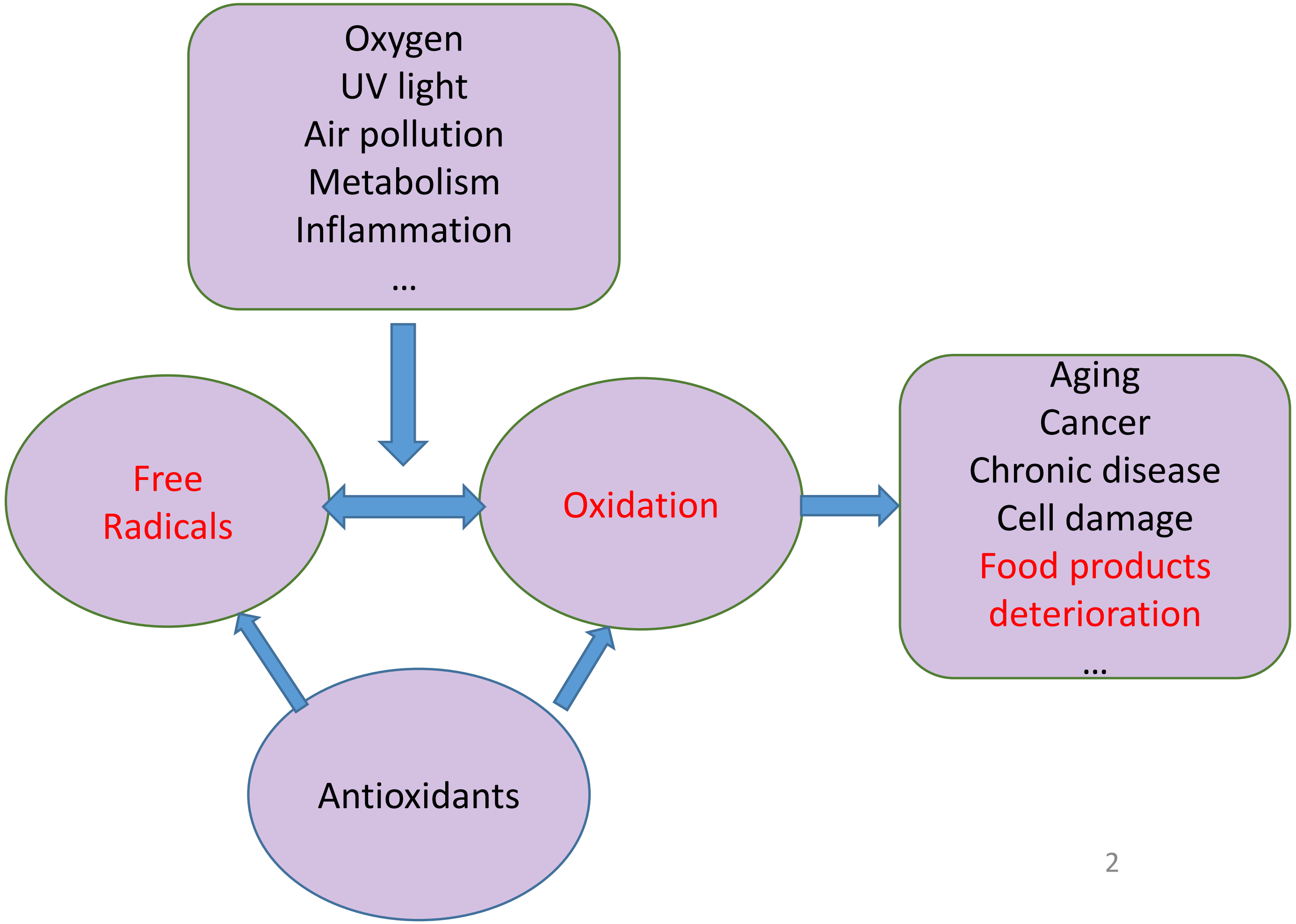
# Antioxidant Classification

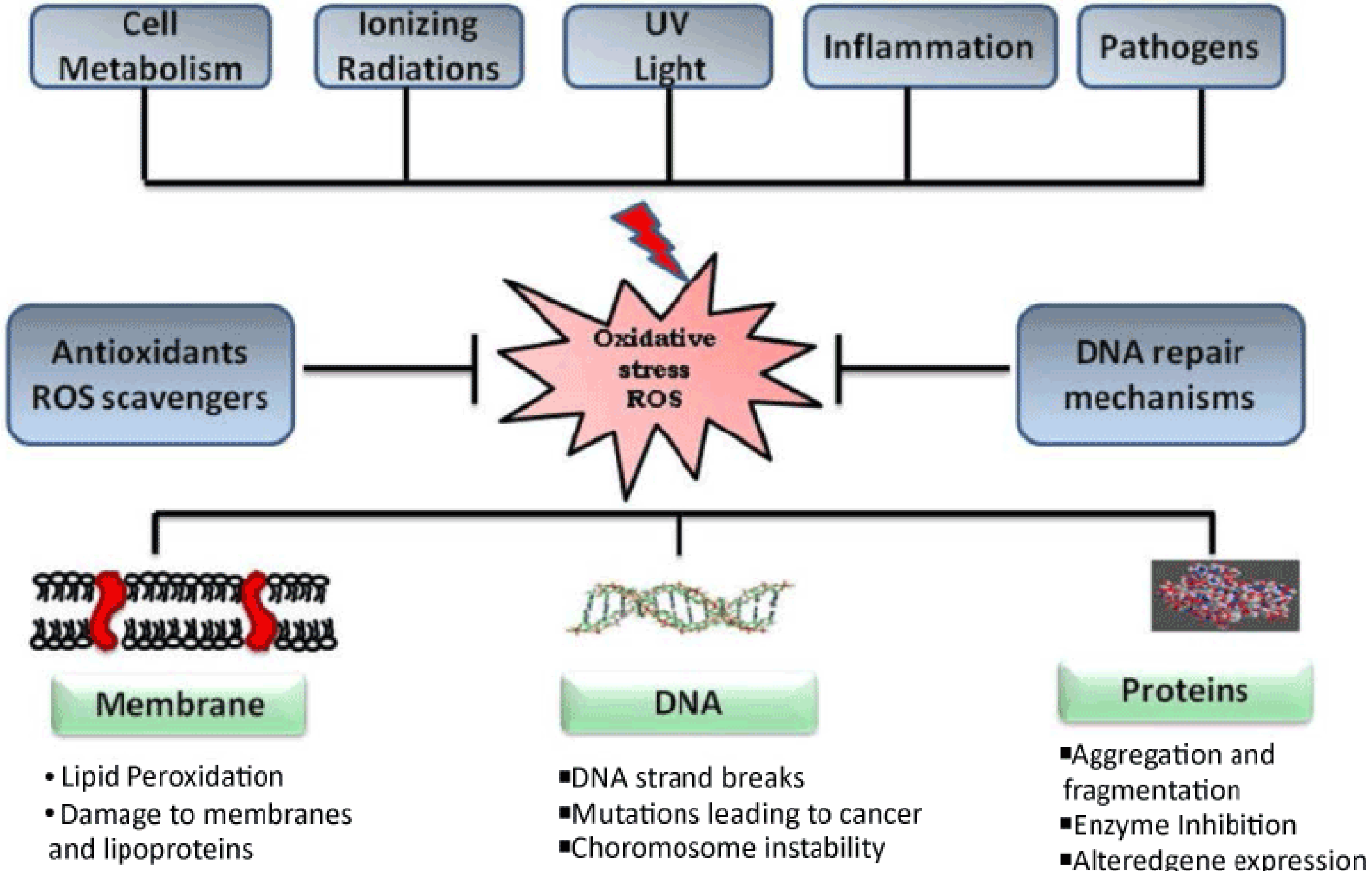
- Substances able to delay or inhibit oxidation reactions
  - Endogenous antioxidants
  - Exogenous antioxidants
  - Antioxidant ingredients



# Endogenous Antioxidants

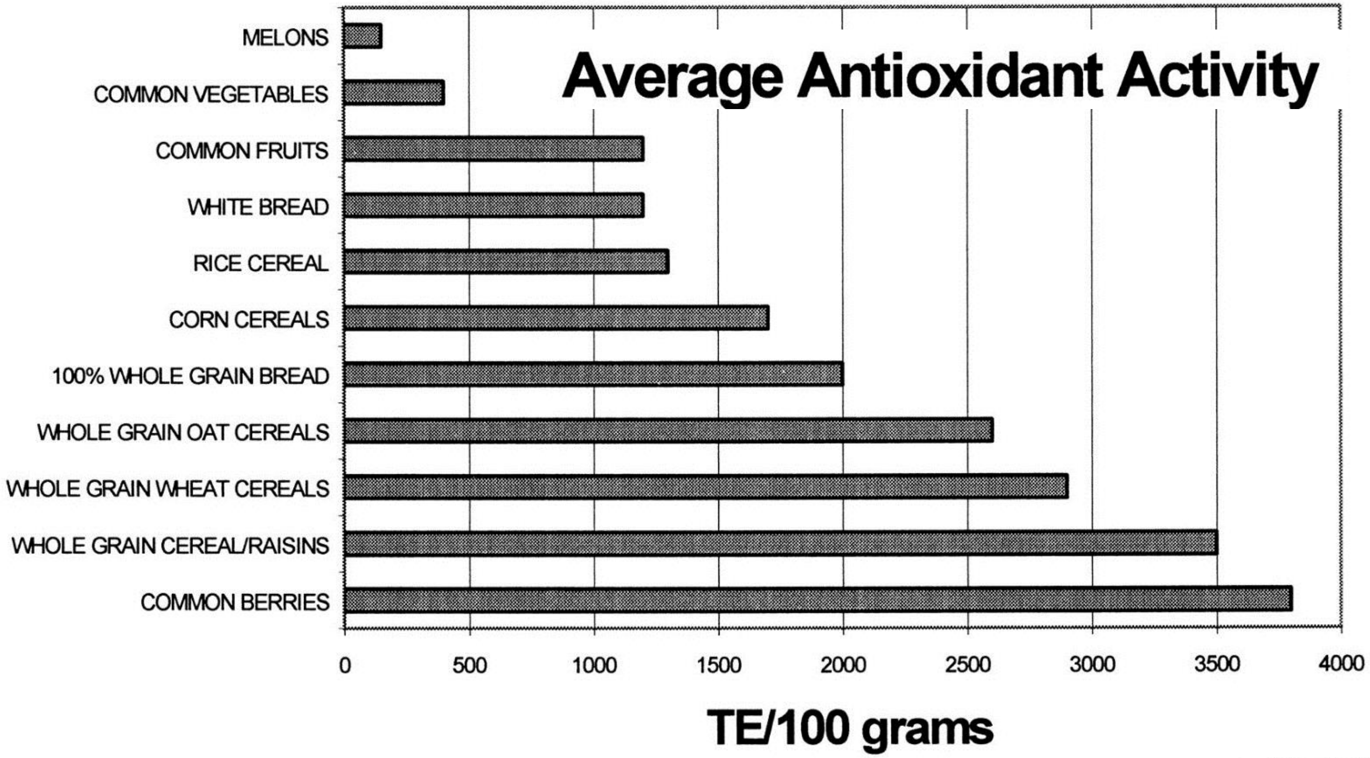
- Produced in human body and living organisms
- Natural defense system to maintain healthy biological systems
- Examples: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), uric acid, glutathione (GSH)




Biol Med, 2014 6:214.

# Exogenous Antioxidants

- Dietary antioxidants
- Mainly as dietary supplements and nutraceuticals, some as food additives
- Examples: Vitamin C, Vitamin E, carotenoids, and phenolics from fruits, vegetables, cereals, etc.



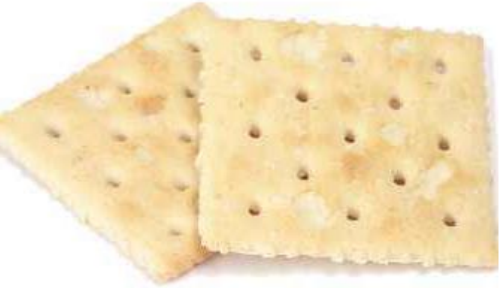
# Antioxidant Ingredient


- **Synthetic antioxidants:**
  - BHA, BHT, TBHQ, PG, EDTA
  - Tocopherols (Vitamin E), ascorbic acids (Vitamin C), citric acid…?? (natural??)
  - Cheap and effective at low ppm
  - Health-related safety concerns, potential toxicity
- **Natural antioxidants:**
  - Phytochemicals and herb extracts (e.g. rosemary extracts, acerola extracts, green tea extracts)
  - Clean label, avoid "chemical sounding" names
  - Complex processing, complicated composition
  - Expensive and less effective
- **Demand for natural and safe antioxidants**


Source: http://www.purenaturalplantextracts.com/

# Antioxidant as Food/Pet Food/Feed Additives

- Processing (e.g. mixing, thermal treatment, lightening exposure, etc.) induces **pro-oxidants** (e.g. $O_2$, radical)
- Oxidation of ingredients leads to **failures of quality features**
  - Alteration in flavors, aroma, texture, color, etc.
  - Generation of toxic products
  - Reduced nutritional profile and shelf-stability
- Antioxidants are commonly added as functional ingredient
- **$Billion** growing market


# Antioxidant Application Example

Stability of Bakery Products (AOM – Days of stability)

| | Treatment (%) | **Pastry** (days of stability) | **Cracker** (days of stability) |
|---|---|---|---|
| **Control** | 0 | 2 | 3 |
| **TBHQ** (tert-butylhydroquinone) | .005 | 2 | 7 |
| | .001 | 3 | 10 |
| | .020 | 4 | 5 |
| **BHA** (butylated hydroxyanisole) | .005 | 8 | 12 |
| | .010 | 21 | 22 |
| | .020 | 27 | 33 |
| **BHT** (butylated hydroxytoluene) | .005 | 5 | 10 |
| | .010 | 10 | 14 |
| | .020 | 19 | 21 |

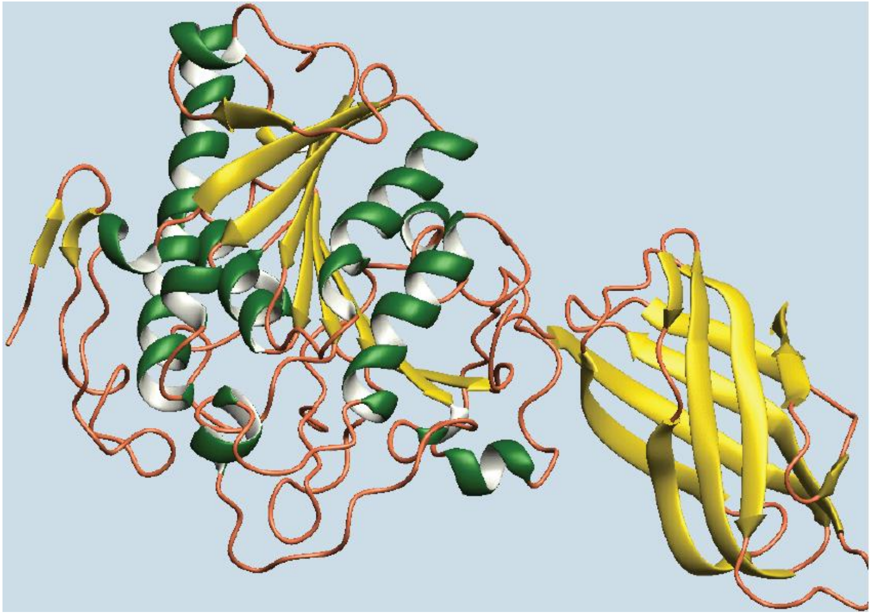


Source: web.utk.edu/~jmount/Classes/515/lipidFeb17.ppt

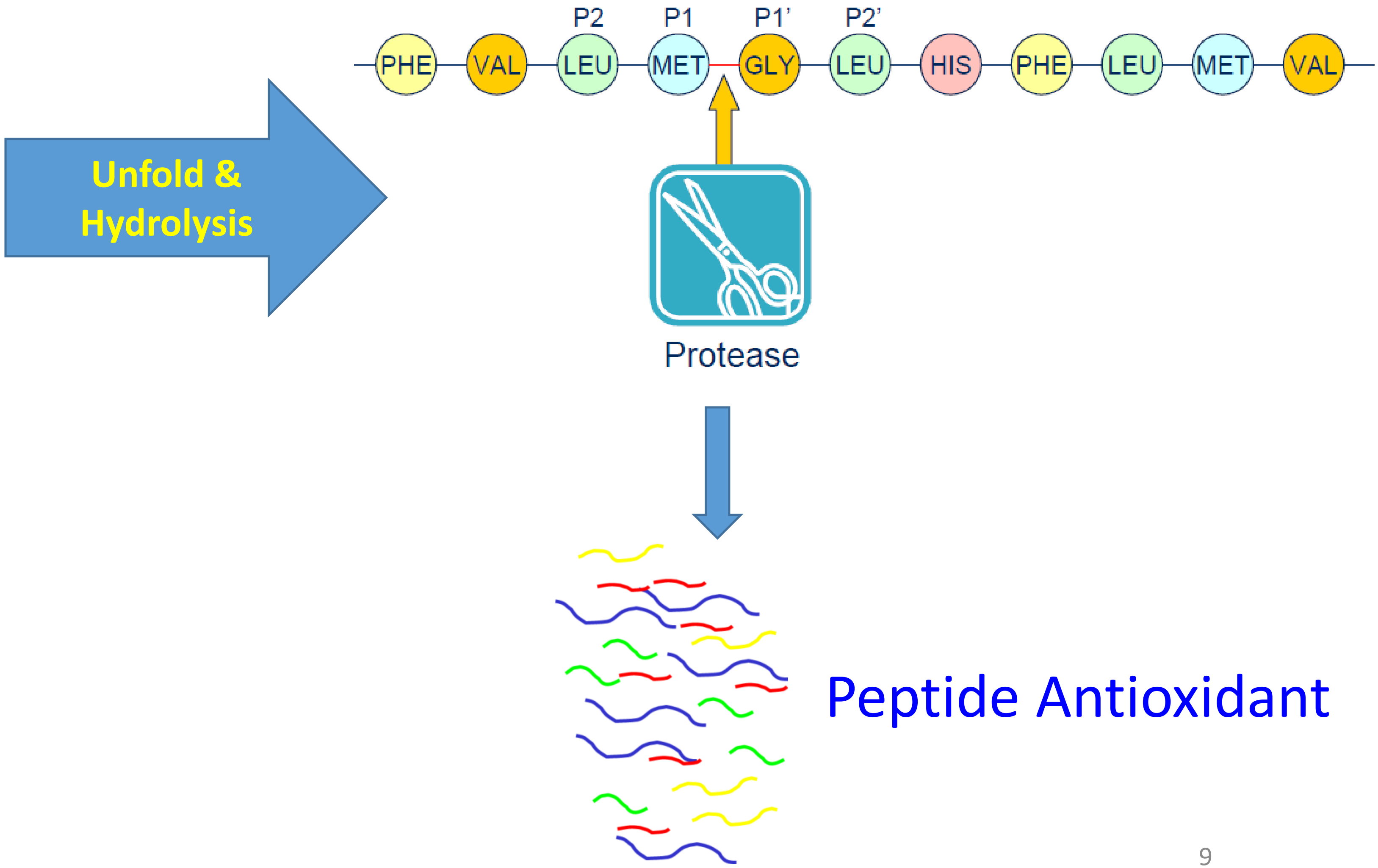
# Cereal & Grain Protein Based Natural Antioxidants

- Increasing safety concerns over synthetic chemicals in foods
- Consumer's preference for naturally derived products
- Abundant renewable grain resource
  - Corn, 345 MMT (14.7 MMT in Kansas)
  - Wheat, 55 MMT (8.8 MMT in Kansas)
  - Sorghum, 15 MMT (7.2 MMT in Kansas)
  - Soybean, 107 MMT (4 MMT in Kansas)
- High protein content of byproducts (DDGS, soybean meal)
- Molecular structural guarantee (e.g., tyrosine, cysteine, methionine)

# Antioxidant Domains in Plant Proteins

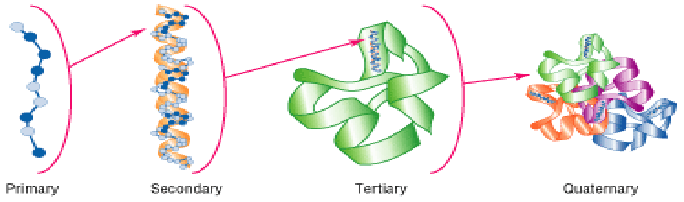


Plant Proteins

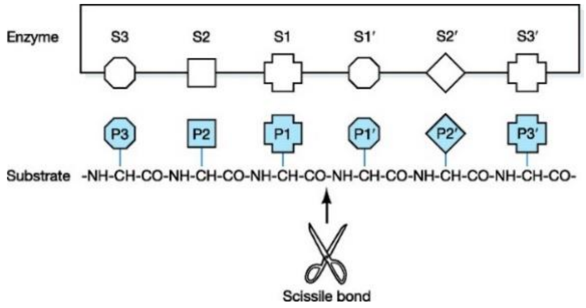


# Enzymatic Hydrolysis

- **Hydrolysis is necessary:**
  - **Unfolding** 3-D globular protein structures
  - **Degradation** into shorter peptides
  - **Exposure** of specific functional groups and structural domains that were buried within the hydrophobic core

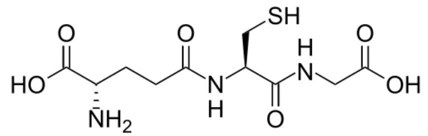


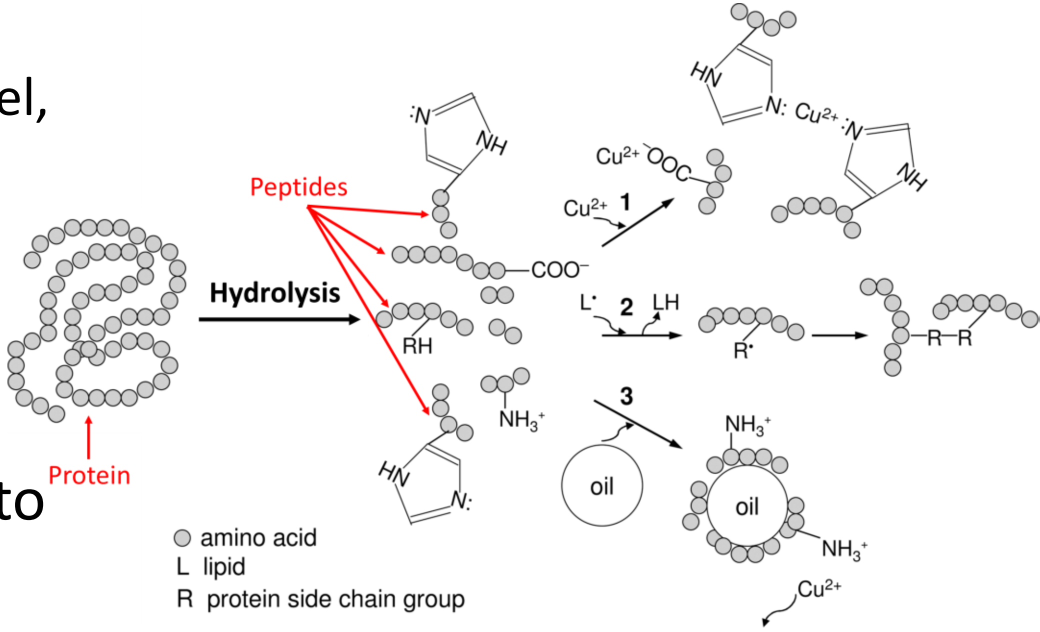

- **Enzymatic hydrolysis** – predominant
  - Cleavage of peptide bond by catalytic protease
  - Mild but highly-efficient:
    - Moderate temperature & pH, preserve nutritional values
    - Limited side reactions, minimal damage
    - High reaction rate
  - Easy to control, consistent products
  - Low cost
  - **Specificity**



# Peptide Antioxidants

- **Sources:**
  - **Naturally-existed**, e.g. glutathione, carnosine, anserine…
  - **Antioxidative peptides** released from parent proteins
    - e.g. corn, wheat, barley, oat, pulses, milk, egg, mussel, fish, etc.
- **Possible mechanisms:**
  - Stabilize free radicals, stop chain reaction
  - Chelation of catalytic transition metals
  - Physical barrier to hinder or minimize the access to targets




Source: Xiong, 2010

# Peptide Antioxidants

- **Multi-functions:**
  - **Efficient**
  - Cost-effective
  - Naturally-derived, **clean label**
  - Safe at high dosage
  - Providing **energy** source & **nutritional profile**
  - **Functionalities** (e.g. solubility, emulsifying, foaming, gelation, oil or water binding capacity)

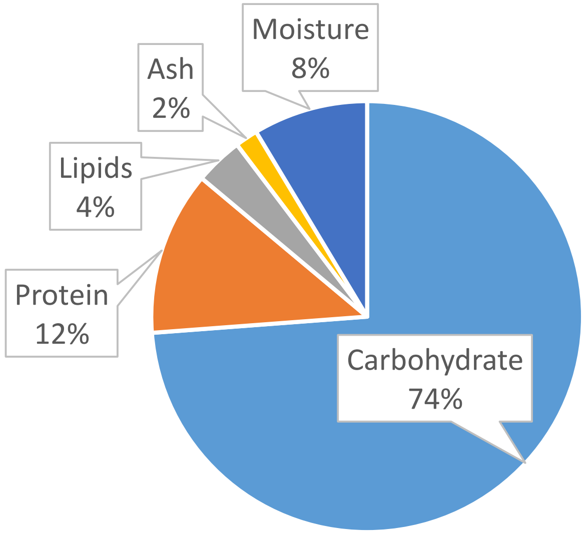
# Sorghum Background

- *Sorghum bicolor* L. Moench is one of the oldest ancient grains
- **Worldwide, 5th** leading cereal crop
- **U.S., 3rd** largest crop (behind maize and wheat)
  - ~15 MMT, globally dominant
- **Sustainable crop**
  - Biological-economic: high biomass yield, short production cycle, efficient utilization of nutrients from soil
  - Tolerance to heat, pests, drought, soil salinity and alkalinity etc.

# Sorghum Grain Composition

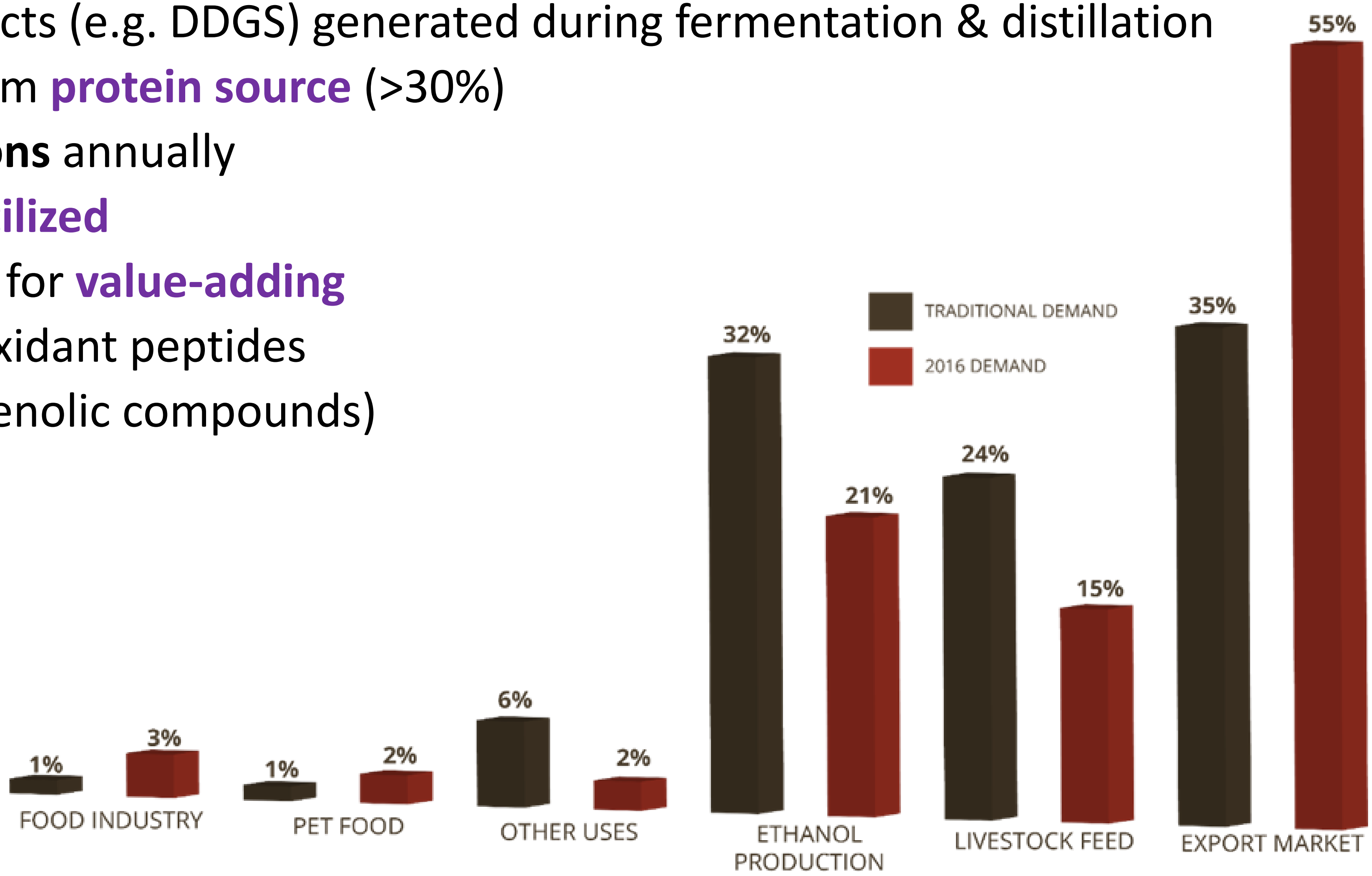
- Overall comparable to **corn** and **millet**

- **Carbohydrate**: endosperm, 74%
  - Higher resistant starch than corn
  - Gluten free
- **Protein**: germ and endosperm, 7-15%
  - **Kafirin**
    - Storage protein (prolamin), 48-70%
    - Almost exclusively localized within endosperm
    - Unique properties, e.g. hydrophobicity & low-digestibility
    - Proven to be bio-active *in vivo* and *in vitro*
- **Crude fat**: germ, 2-4%
- **Minerals**, vitamins, phytochemicals: bran and germ
  - Higher phenolic content than other major cereals



# U.S. Sorghum Utilization

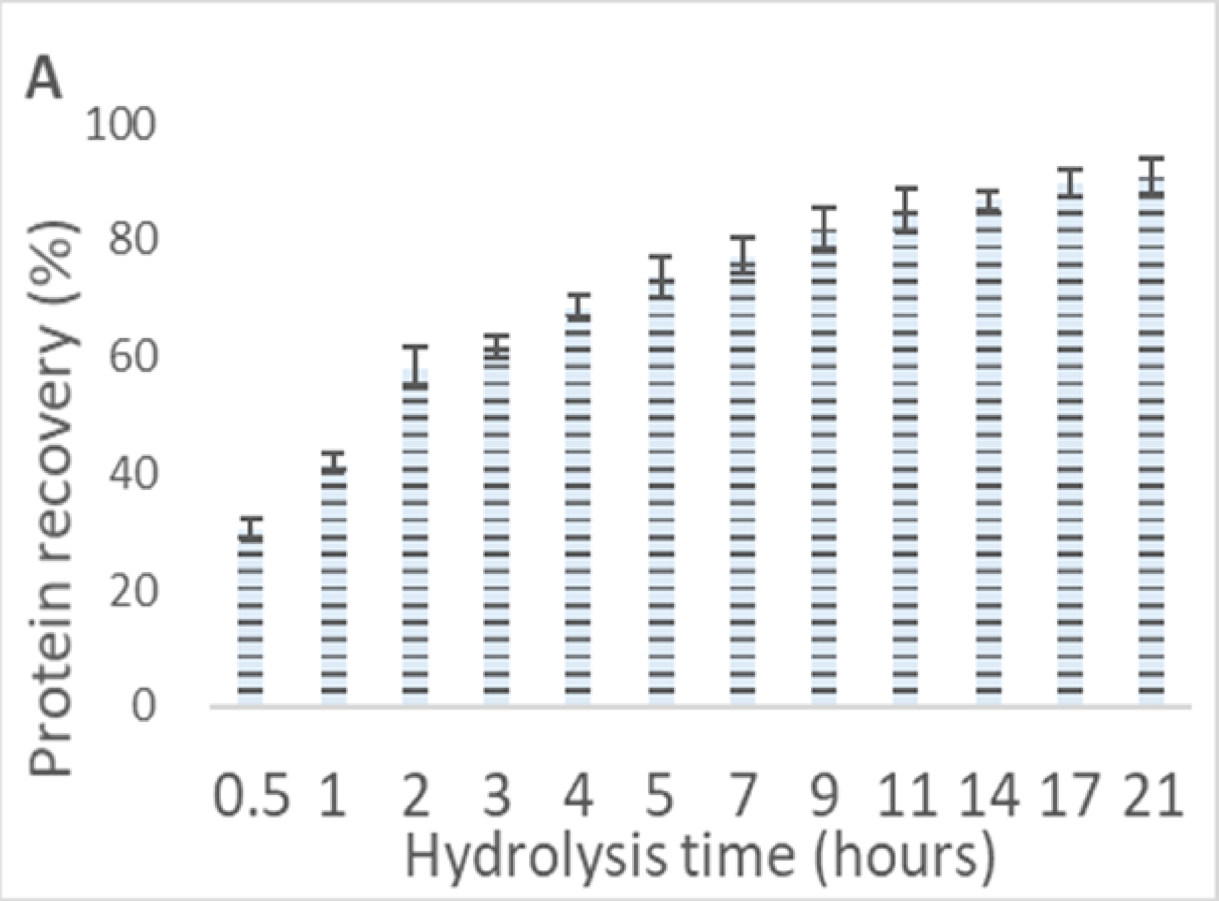
- Mainly used for **livestock feeds**, and **biofuels**
  - By-products (e.g. DDGS) generated during fermentation & distillation
  - A premium **protein source** (>30%)
  - > **450k tons** annually
  - **Under-utilized**
  - Potential for **value-adding**
    (e.g. antioxidant peptides
    and phenolic compounds)

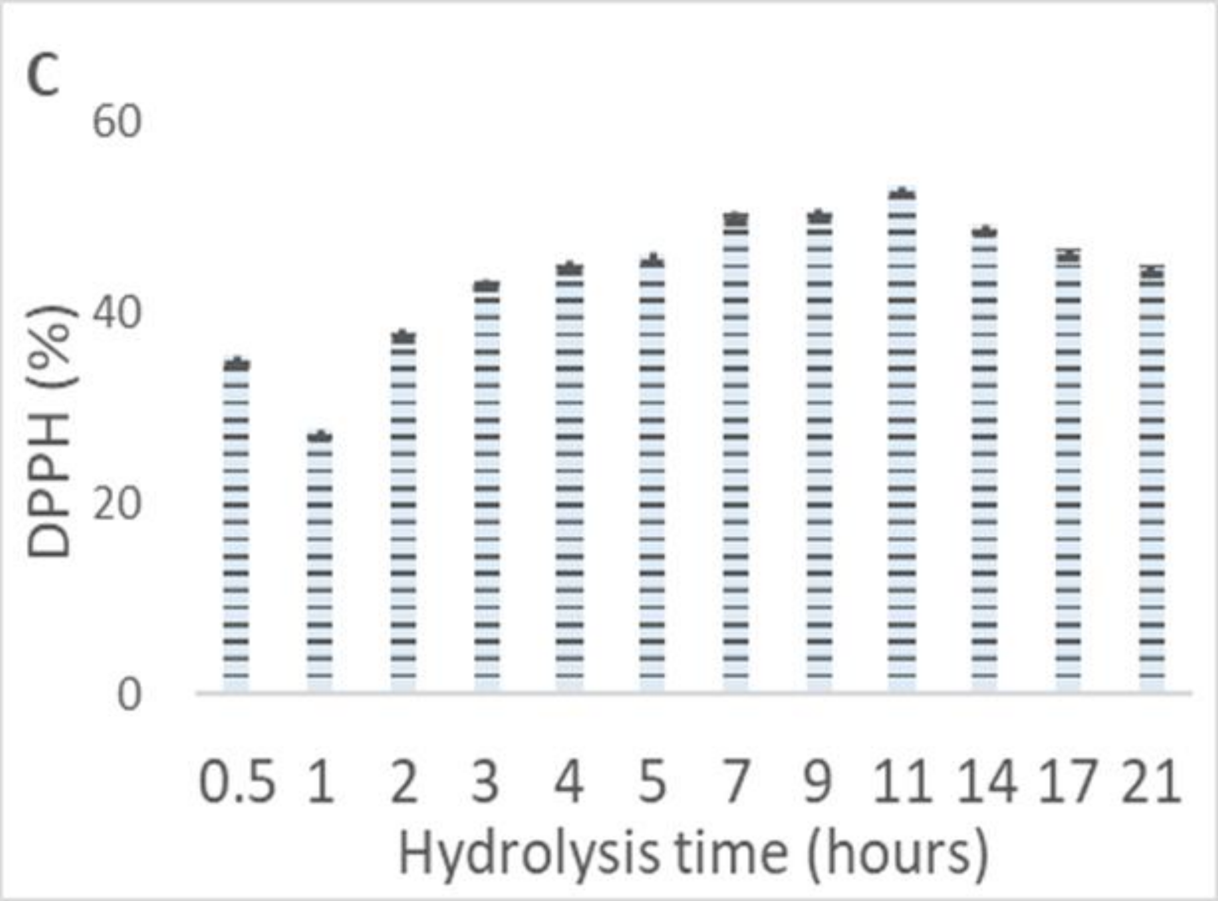





Source: sorghumcheckoff.com

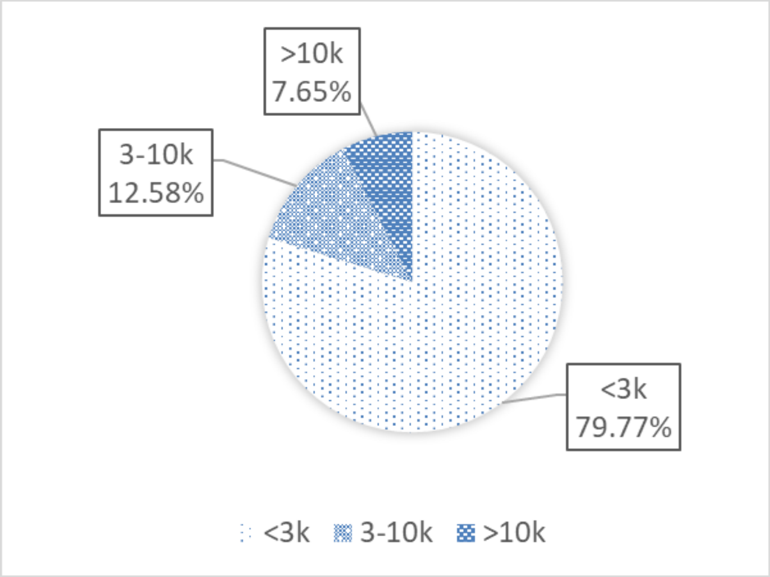
# Effect of Hydrolysis Time

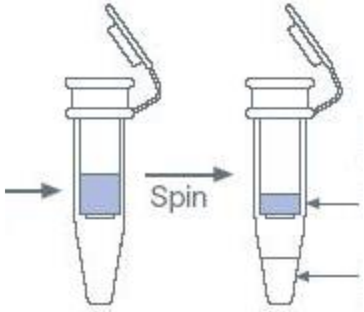


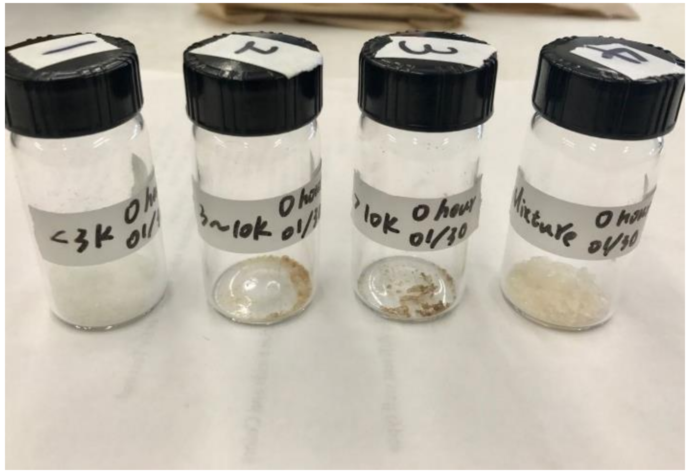



# Fractionation & Purification – Ultrafiltration

- To study <u>molecular weight–antioxidant activity</u> relationship
- To fractionate hydrolysates according to MW
  - **Centrifugal tubes** with 10k & 3k Da MW cut-off membranes
  - Different MW ranges: <3k, 3-10 k, >10k, Mix
- Distribution: majority fell into **<3k** range
- Tested for antioxidant activity

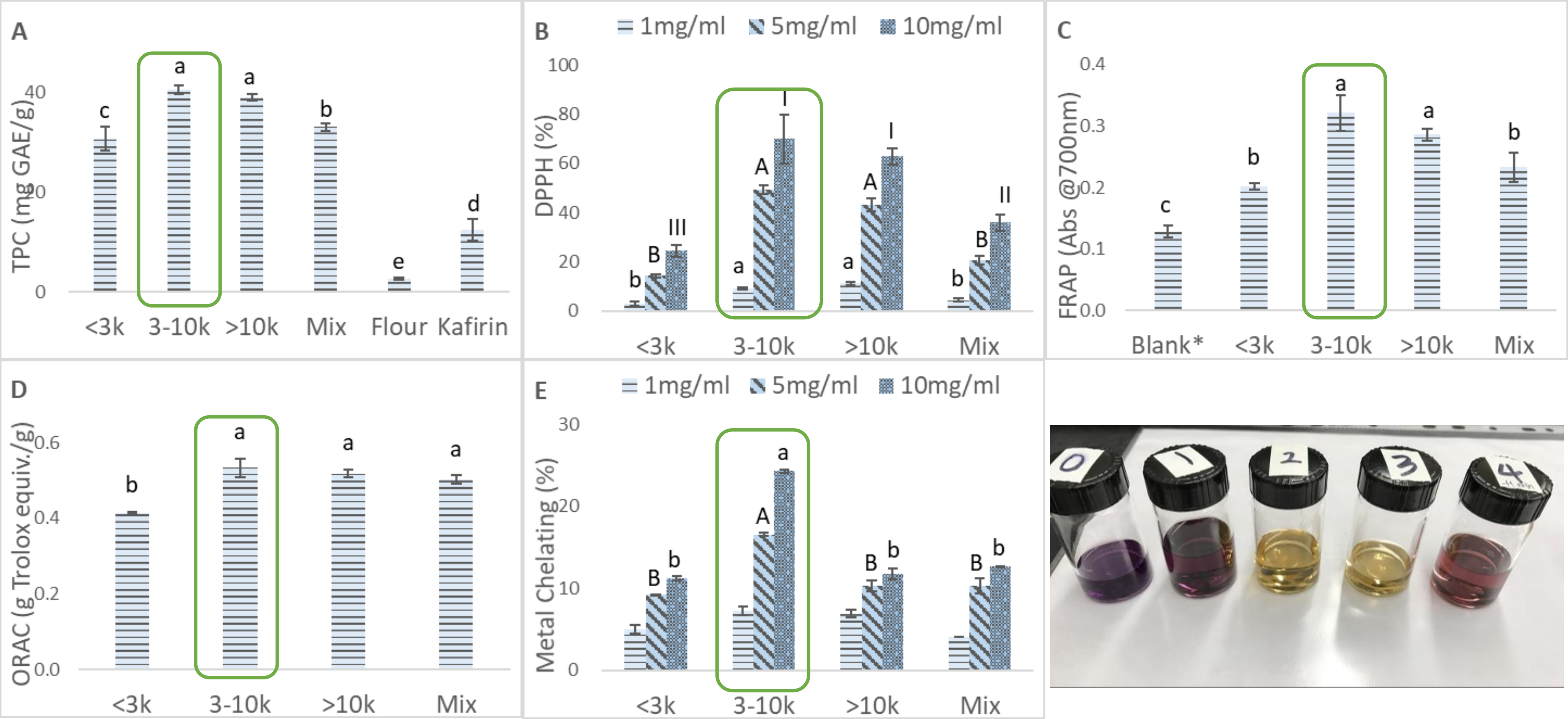


# Fractionation & Purification – Ultrafiltration

- ***In vitro***: TPC, DPPH%, ORAC, FRAP, Metal chelating%
- **Enhanced TPC** compared to sorghum flour or sorghum protein
- Medium-sized (**3-10k Da**) fraction yielded a higher activity compared to other fractions and original hydrolysates

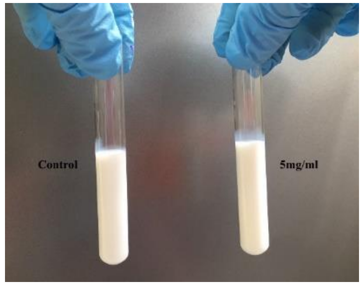


# Oil/lipid Inhibition – in Emulsion Model Systems

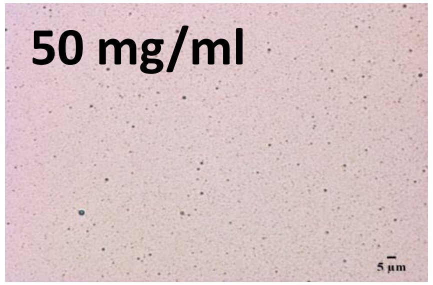
- Neutrase 3–10k 50 & 100 mg/ml oil vs. blank control
- **Emulsion stability** was not significantly decreased
- Average inhibition rate during 14-day incubation at 37 °C:

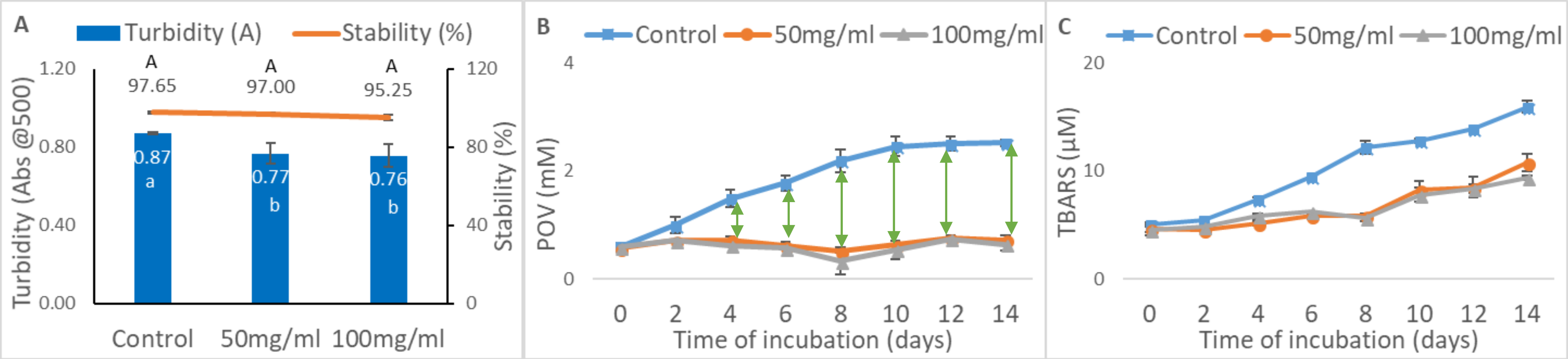
POV: **77.14 ± 13.81%** (50 mg/ml) **76.41 ± 13.81%** (100 mg/ml)

TBARS: **54.34 ± 9.78%** (50 mg/ml) **59.91 ± 13.51%** (100 mg/ml)



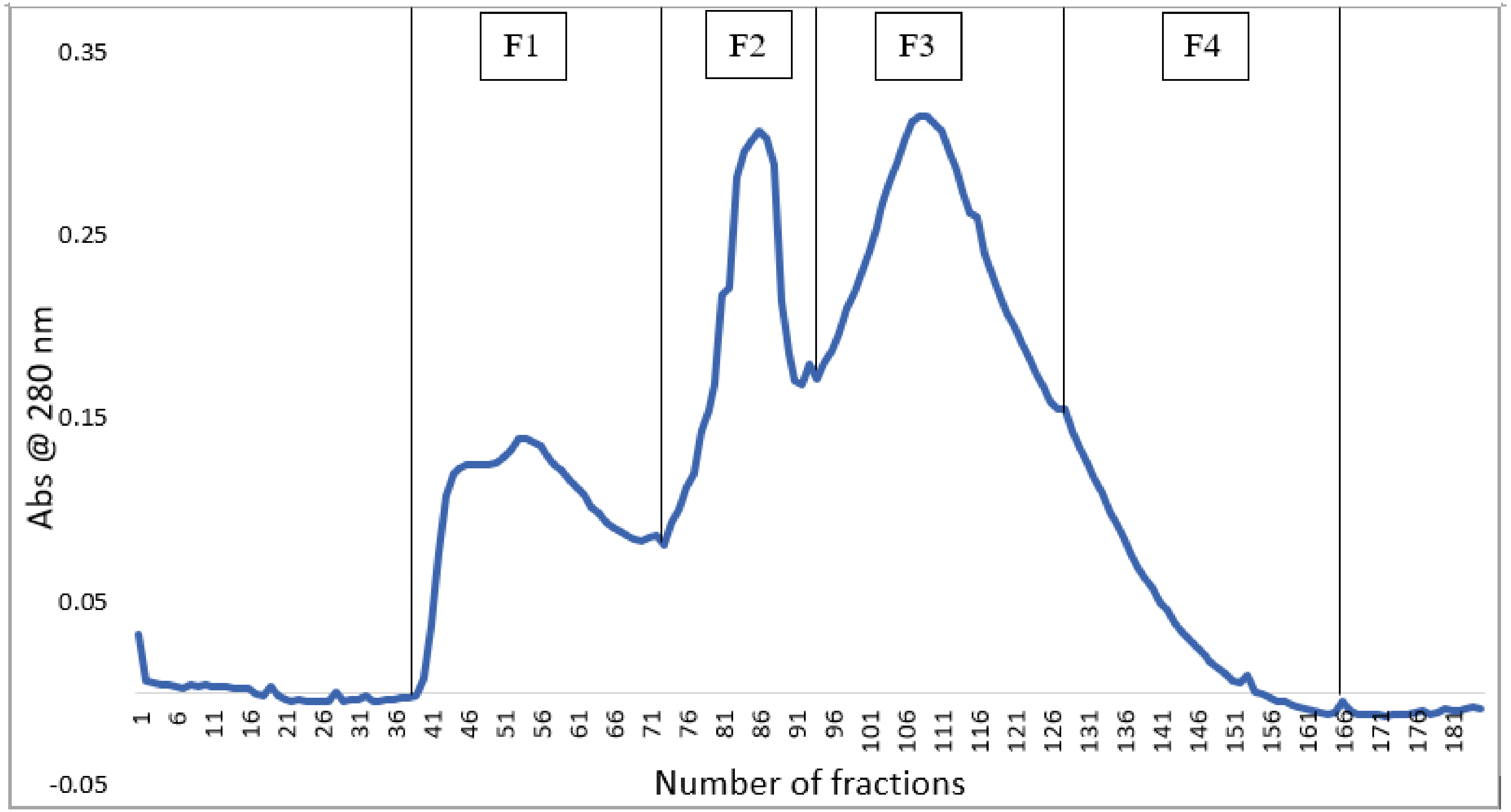




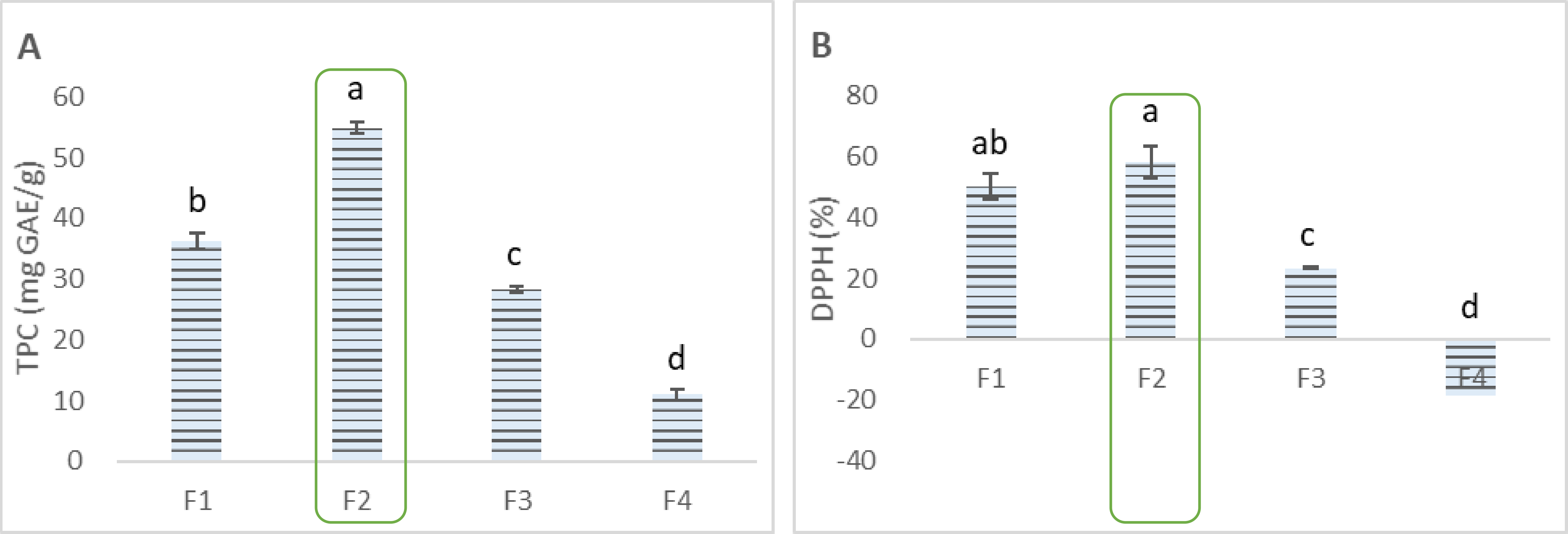
# Characterization & Identification – Gel Filtration

- Neutrase 3-10k Da showed higher activities in previous assays
- To further fractionate 3-10k Da fraction based on **MW profile**
  - F1 (largest) – F4 (smallest): collected, freeze-dried, analyzed



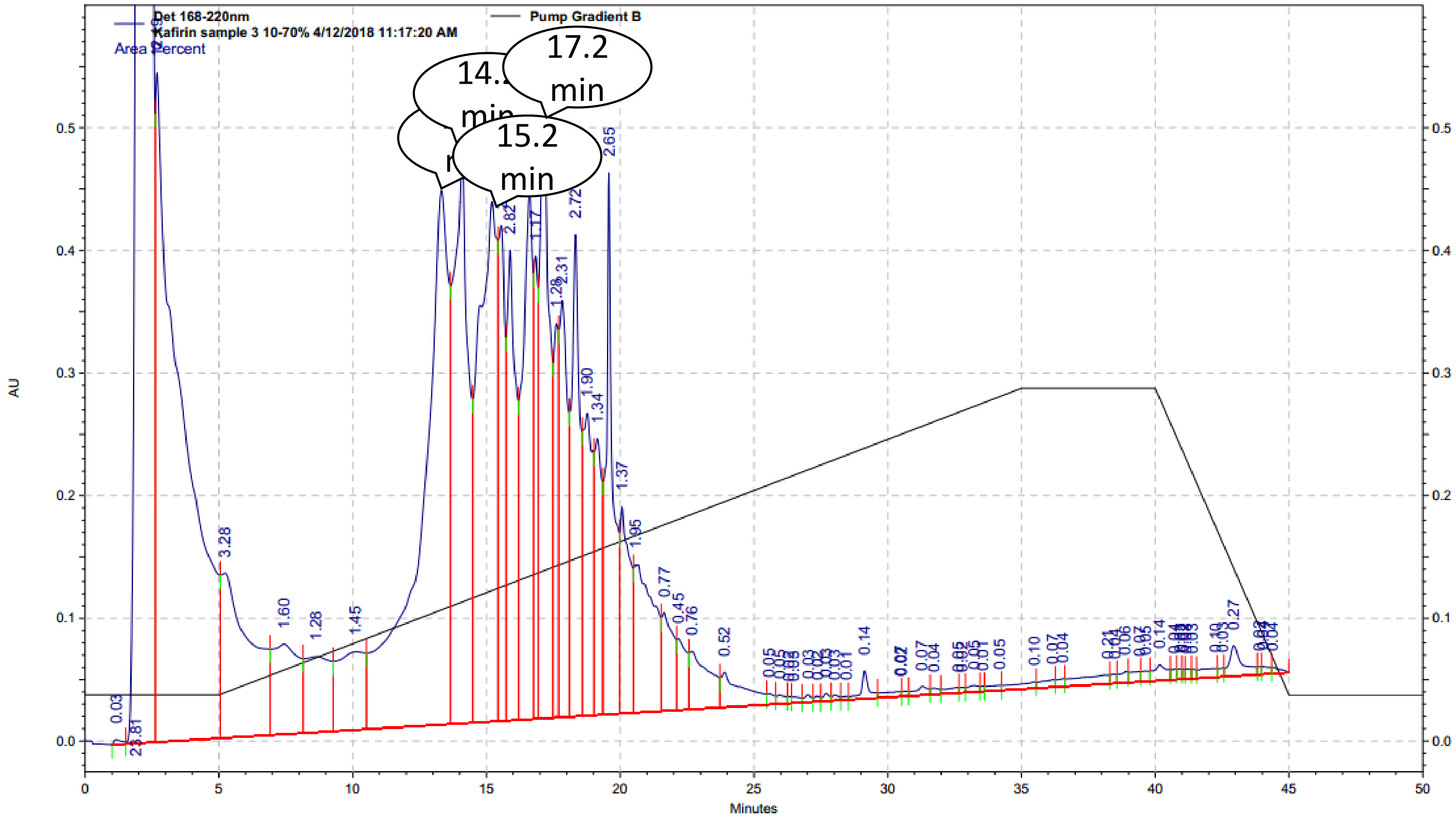
# Characterization & Identification – Gel Filtration

- **F2** exhibited significantly stronger TPC and DPPH%
- Selected for peptide sequence identification



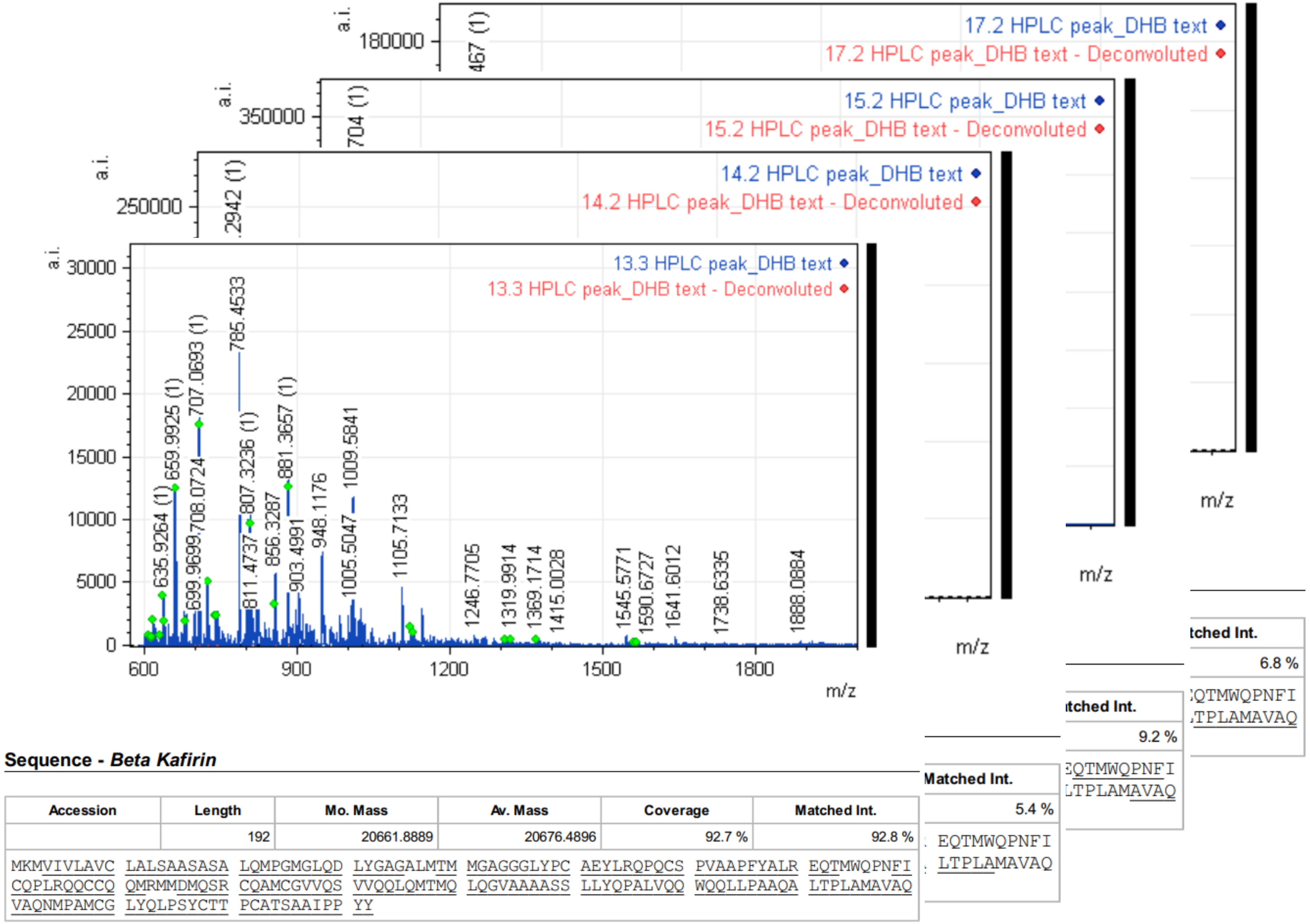
# Characterization & Identification – RP-HPLC

- Gel filtration F2 Neutrase 3-10k Da – **RP-HPLC** peak collection



# Characterization & Identification – MALDI-TOF/TOF MS

➤RP-HPLC peaks **– MALDI-TOF/TOF MS** sequences identification




**Sequence - *Beta Kafirin***

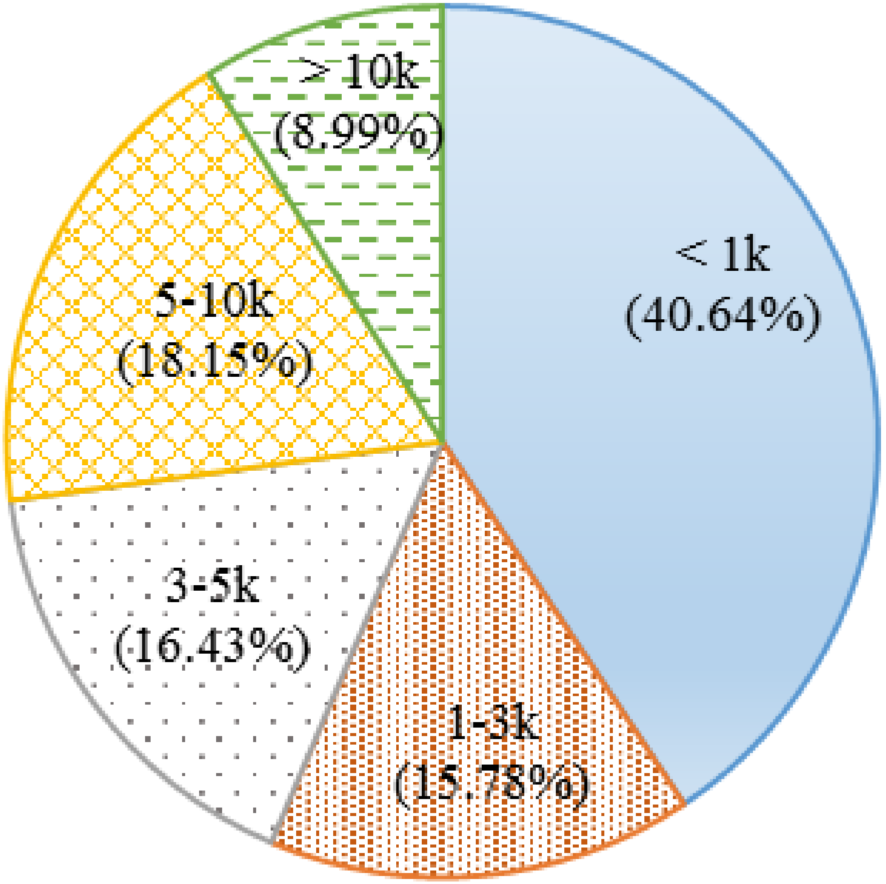
| Accession | Length | Mo. Mass | Av. Mass | Coverage | Matched Int. |
|---|---|---|---|---|---|
| | 192 | 20661.8889 | 20676.4896 | 92.7 % | 92.8 % |

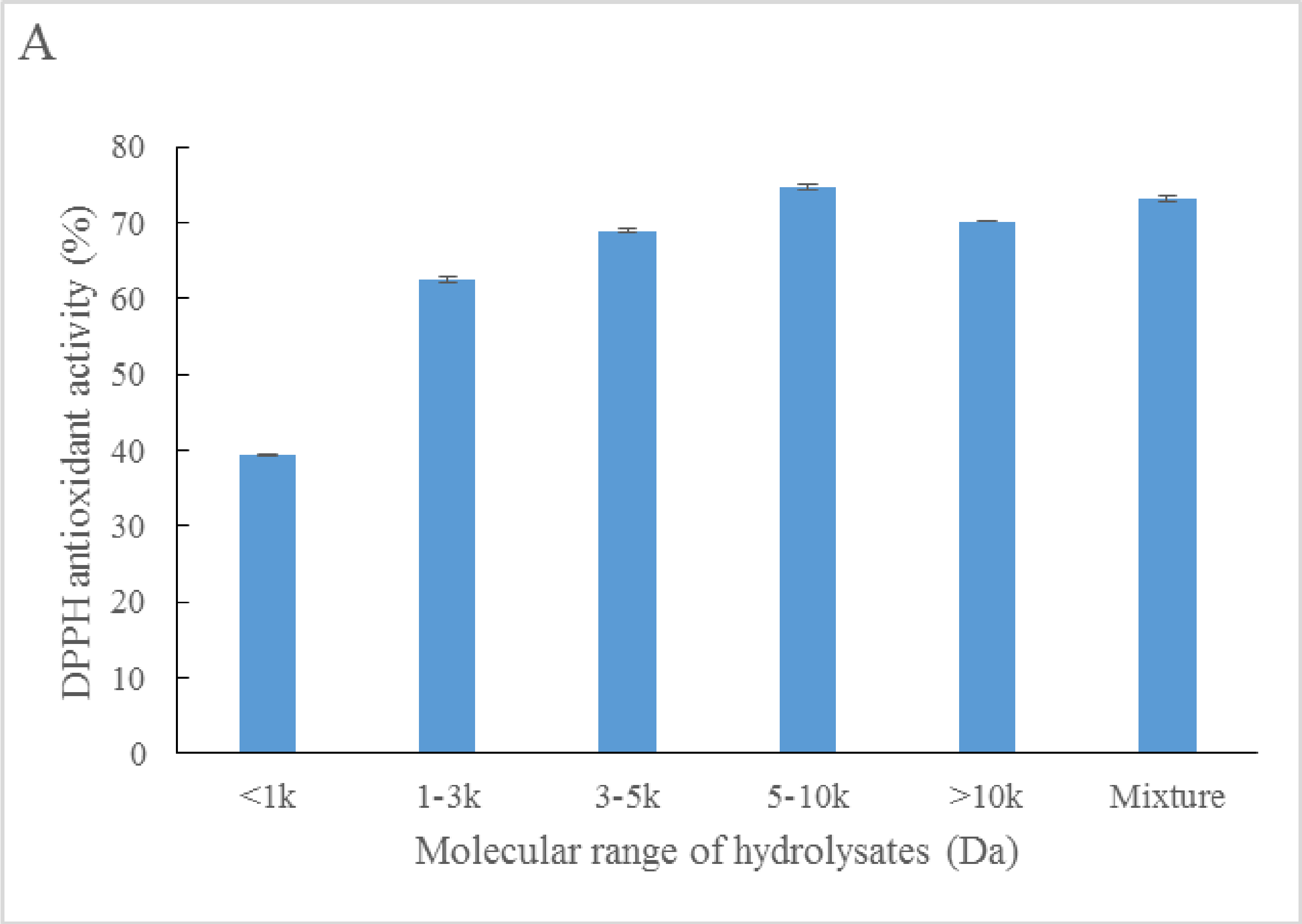
MKMVIVLAVC LALSAASASA LQMPGMGLQD LYGAGALMTM MGAGGGLYPC AEYLRQPQCS PVAAPFYALR EQTMWQPNFI
CQPLRQQCCQ QMRMMDMQSR CQAMCGVVQS VVQQLQMTMQ LQGVAAAASS LLYQPALVQQ WQQLLPAAQA LTPLAMAVAQ
VAQNMPAMCG LYQLPSYCTT PCATSAAIPP YY

# Opportunities for Corn Byproducts

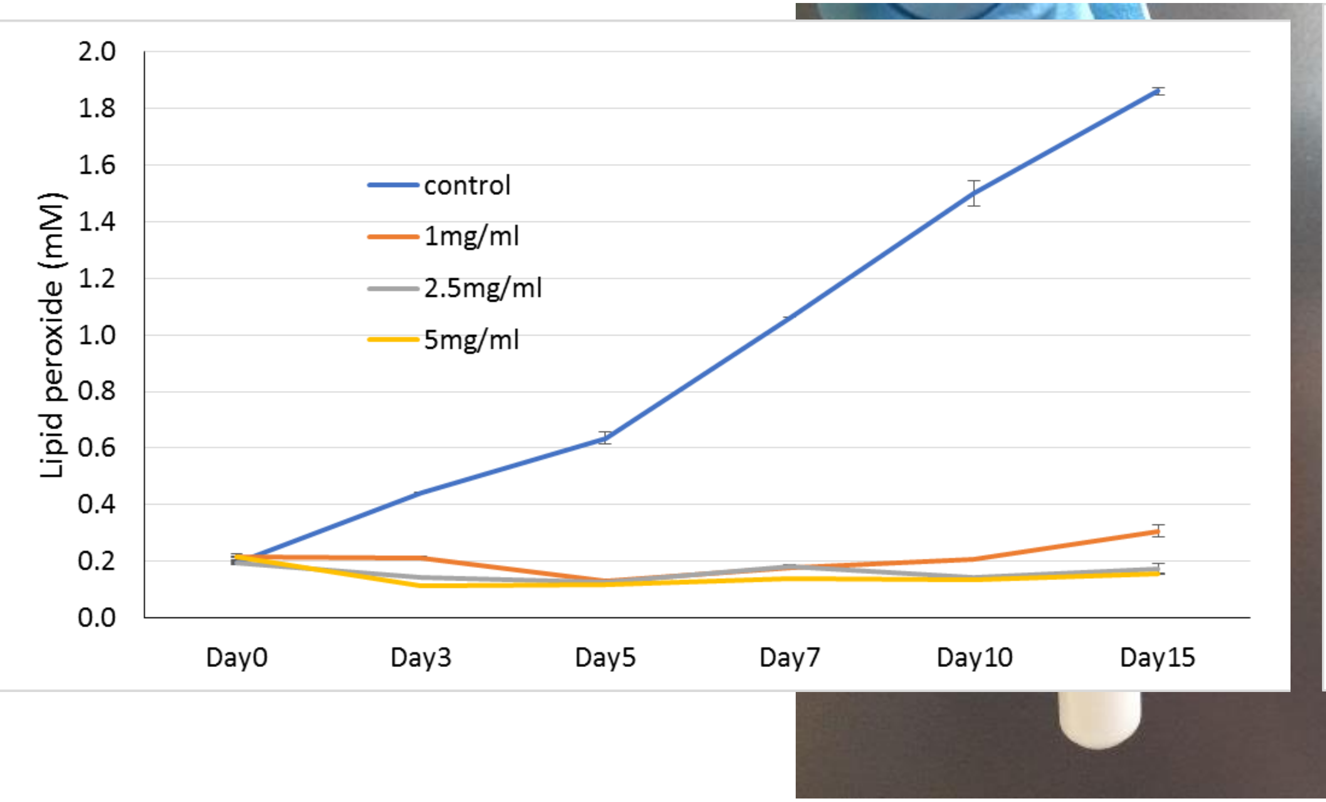
- Carbohydrate → Fuel ethanol
- Protein/phytochemicals → Natural antioxidants
- Remaining → Feed and others

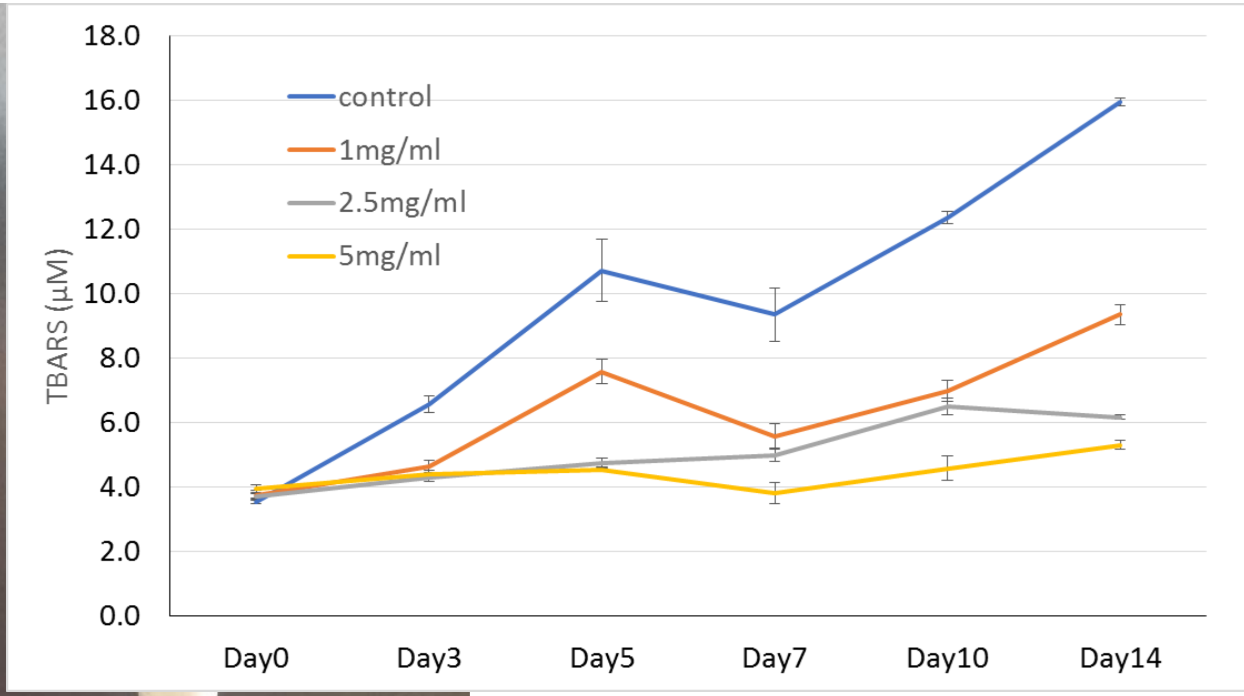
# Ultrafiltration of Corn Peptide Antioxidant



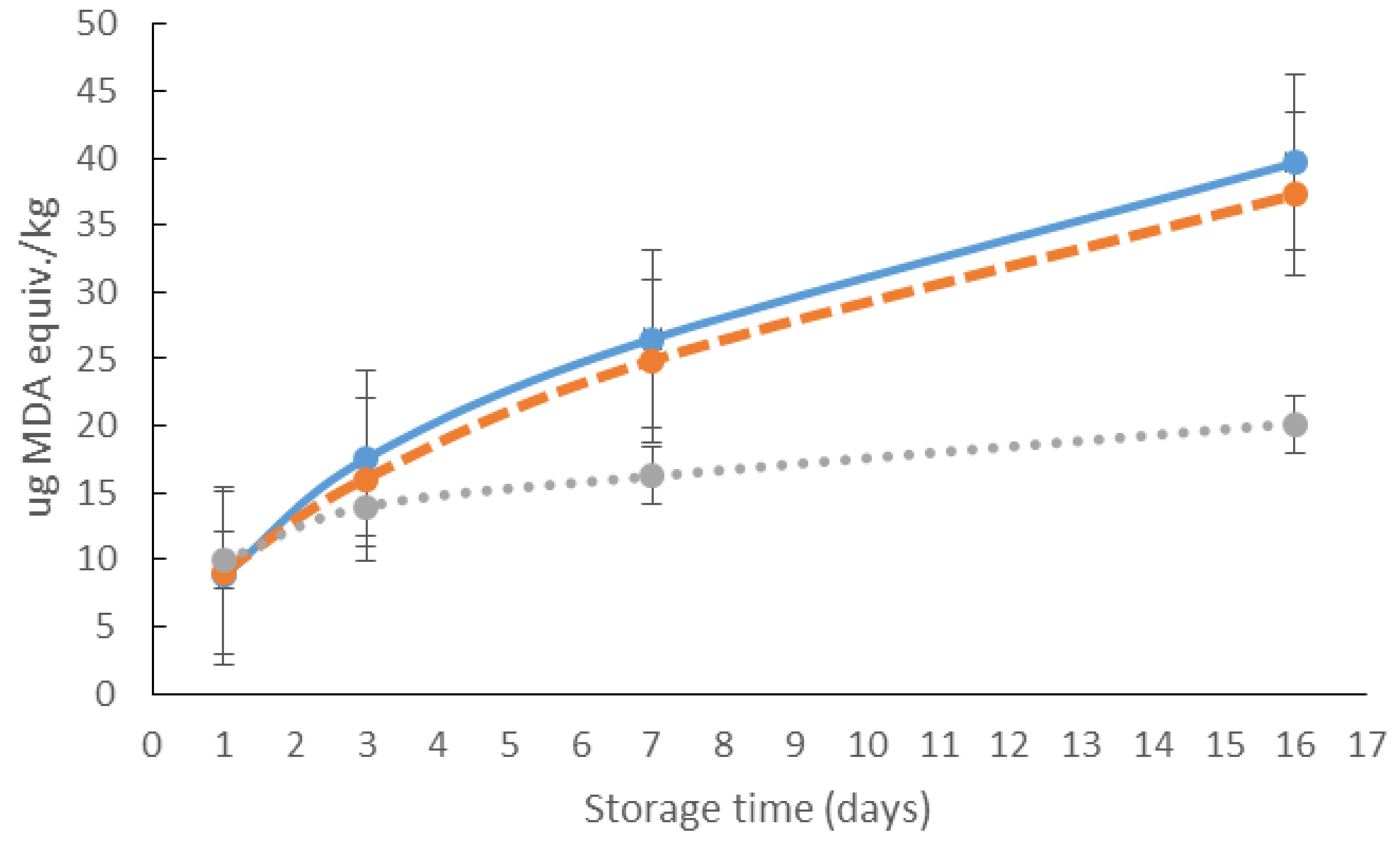



# Corn Antioxidant Performance in Oil/water Emulsions



# Corn Antioxidant Performance in Ground Meat Systems




# Take-home Message

- Plant proteins (e.g., corn, sorghum) are potential source for antioxidant peptides production.
- Antioxidant activity depends on types and amount of proteases, reaction parameters and hydrolysate composition.
- Peptide antioxidant showed good performances in emulsion, meat, oil/fat systems.
- Ongoing research to evaluate performances in pet food and other systems

# Acknowledgements


- Kansas Corn Commission for corn antioxidant research
- Grain Science & Industry department for sorghum antioxidant reserach
- KSU Biotechnology/Proteomics Core Lab for MALDI-TOF MS
- GPC and ADM for samples


KANSAS CORN COMMISSION