Medium-Chain Fatty Acids to Inhibit Yeast and Mold Spoilage in Pet Treats

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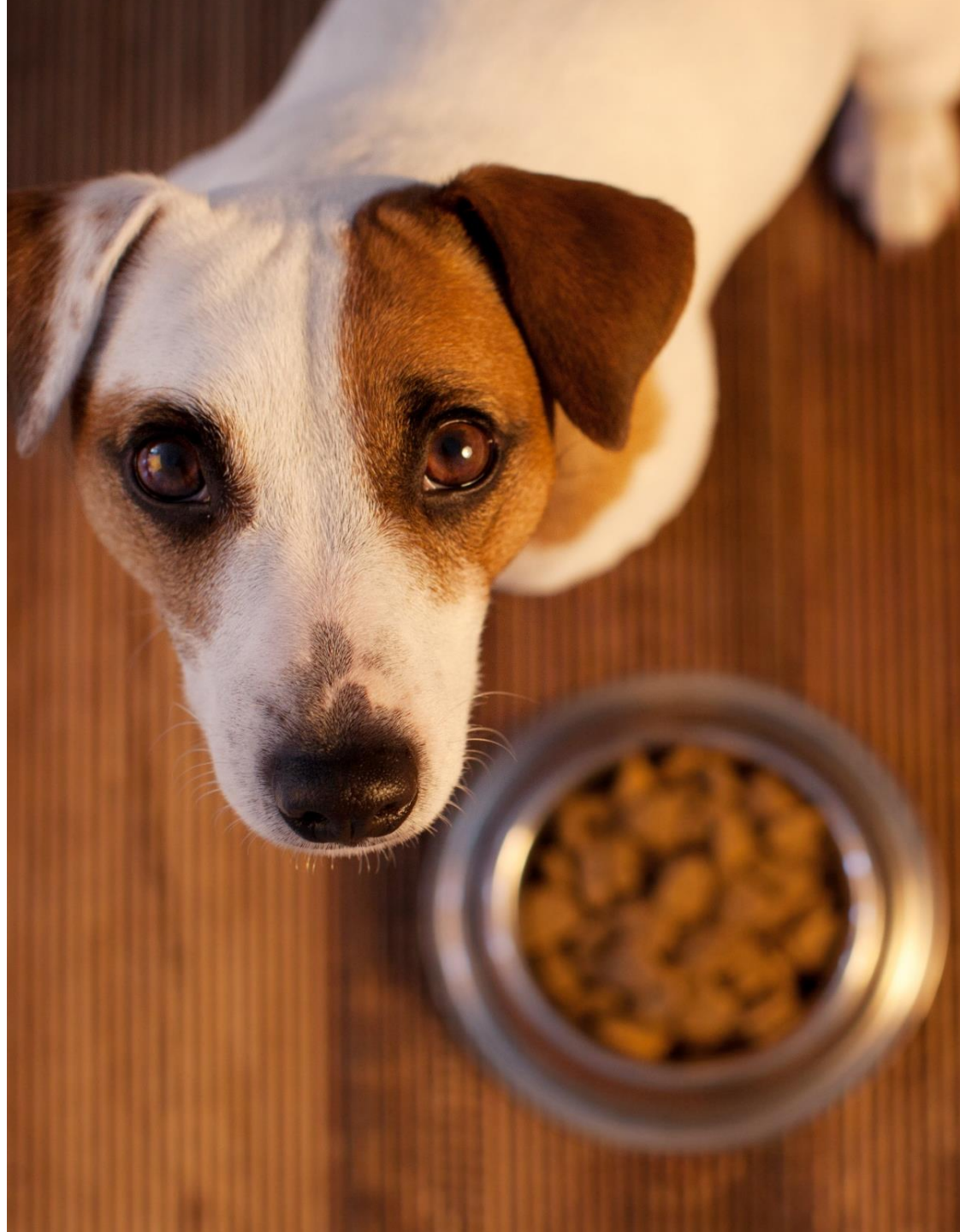
Agenda

- Spoilage Risks
- Testing Methods
- MCFA Mode of Action



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Petfood Industry Market Trends

CLEAN LABELS

- Limited ingredient diets
- Limited processing (raw, fresh, frozen, freeze-dried)
- Environmentally friendly, natural, novel ingredients

PREMIUMIZATION / HUMANIZATION

- Trickle down effect from human foods
- “What’s good for me is good for my pet”
- Culinary experience

SUSTAINABILITY

- Trends to action
- Packaging
- Novel ingredients
- Increased value of rendered fats and proteins

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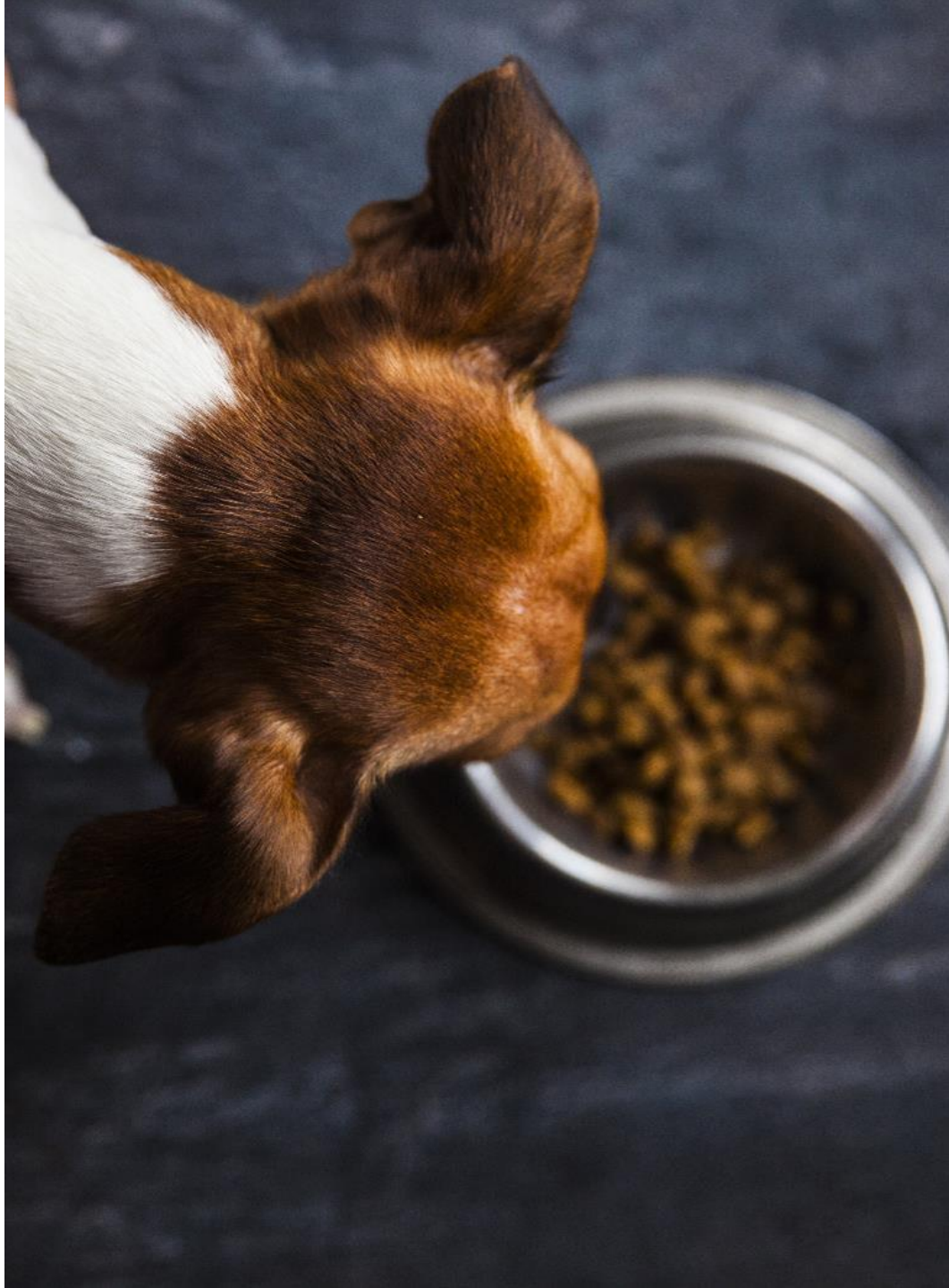


Spoilage Risks

Food Safety Microorganisms

Fungi

- Immobile
- Spread by spores
- Molds and Yeasts
  - *Zygosaccharomyces*
  - *Eurotium/Aspergillus spp.*

Bacteria

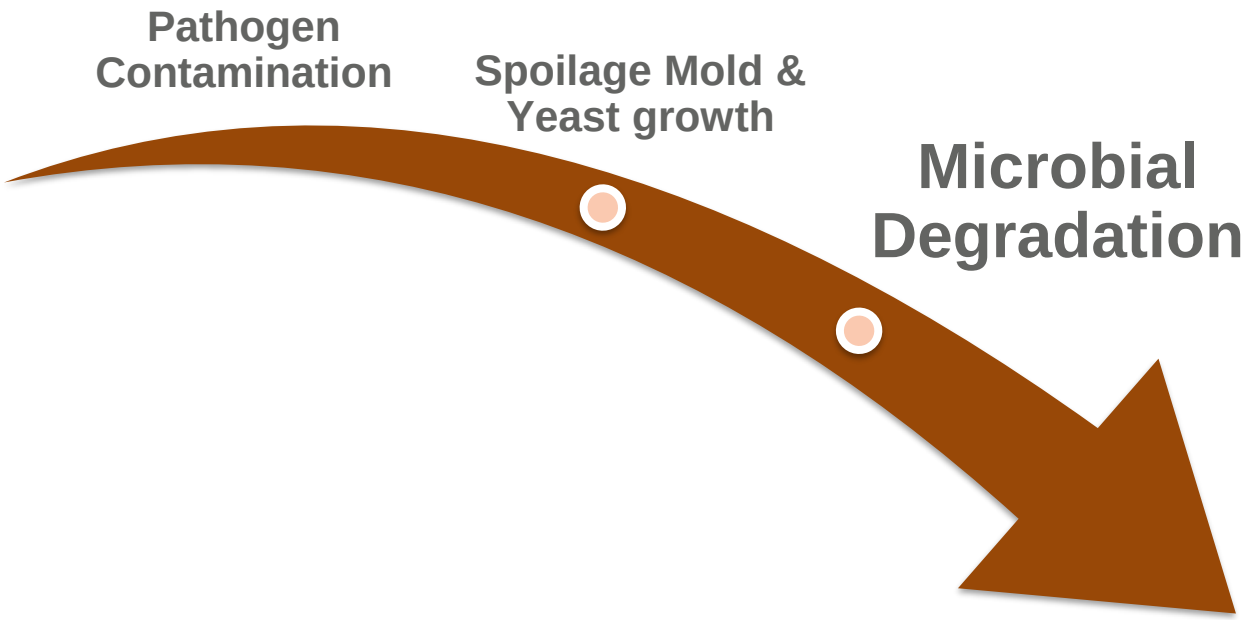
- Mobile
- Not visible with naked eye
- Friendly and non-friendly
  - *Salmonella spp.*
  - *L. monocytogenes*

What is Microbial Spoilage

Deterioration of raw materials, unfinished ingredients, or finished pet products by **naturally occurring or contaminant microbes**

- Color, Odor, and Texture Changes
- Pockets Of Gas and Package Swelling
- Vitamin and Amino Acid Instability
- Elevated Free Fatty Acid Content
- Reduction of Nutrient Value
- Reduced Shelf-life
- Pet Rejection
- Recall

Inherent Product Risks



Many product forms are at increased risk due to high **water activity**

Quality and Performance Compromised

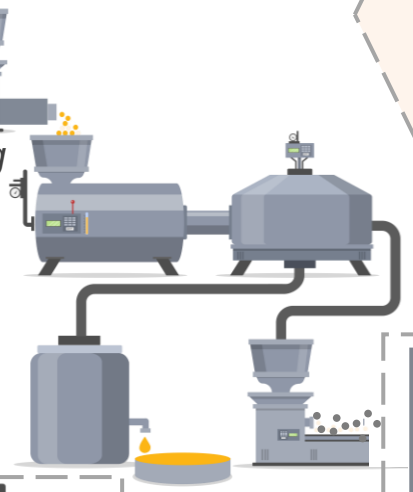
- Reduced shelf-life
- Decline in nutritive value
- Negative color and texture effects
- Reduced palatability
- Pet rejection

Raw Materials and Unfinished Ingredients
food safety pathogen & re-contamination prevention

Finished Petfoods and Pet Food Products
shelf-life extension of finished petfood products

Viscera & Offal Raw Materials

Rendering Plant



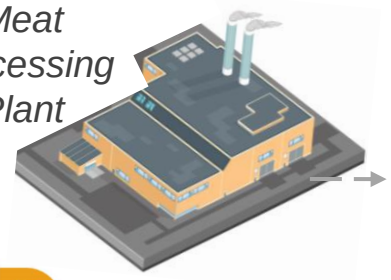
Rendered Meal Transport

Rendered Fat Transport

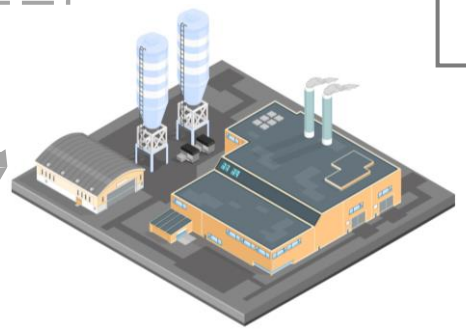
Export Meal

Meat Ingredient Collector

Meat Processing Plant



Meat Slurry and Freeze-Dried or Dehydrated Meats



Finished Petfoods and Petfood Products

Distribution Transport

- Wet Petfood: Raw, Fresh Refrigerated, Canned Diets
- Indulgent Treats
- Natural Chews, Dental, Functional Treats
- Dry Kibble Diets
- High Meat Treats and Toppers
- Bird Seed, Fish Flake, Small Animal Diets

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Microbial Risk Management

Process Controls

- Process flow – contamination post kill step
- Thermal regulation
- Product formulation
- Package integrity

Effective microbial risk management requires a complete hazard analysis on the entire supply chain and production process, with preventative controls put in place

Sanitation Controls

- Equipment and employee hygiene

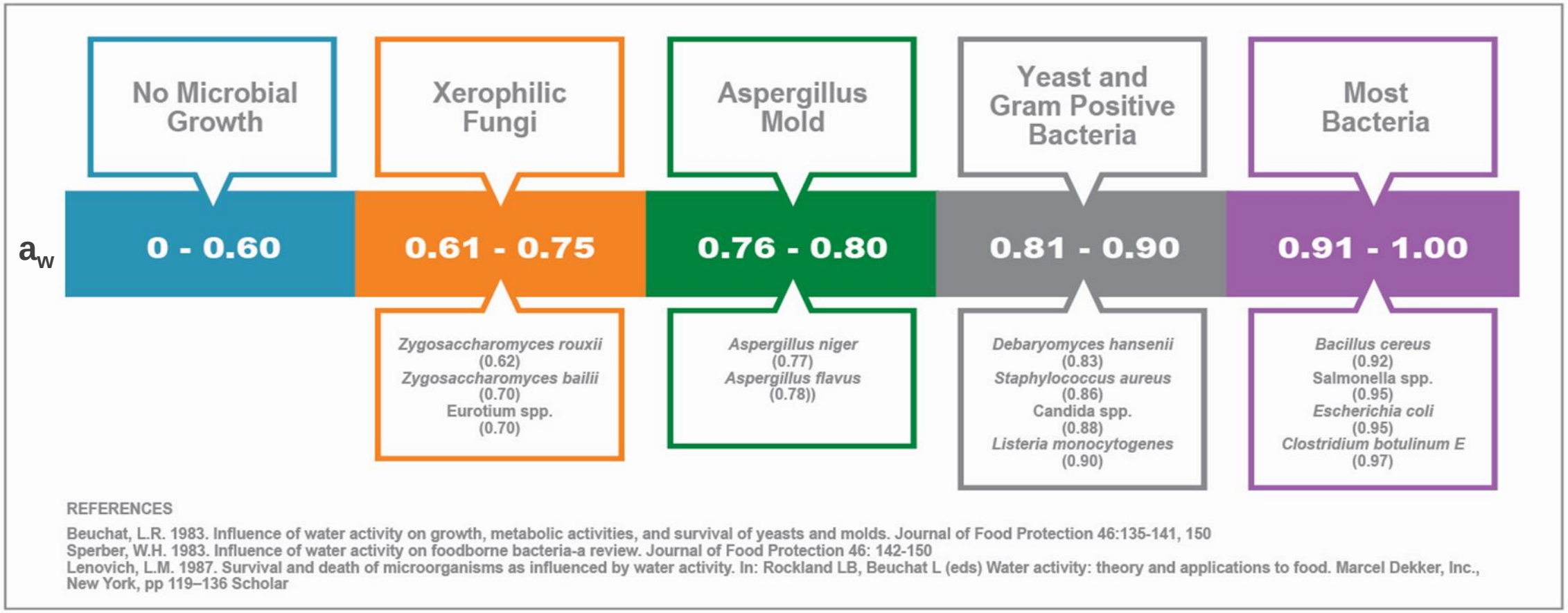
Supply Chain Controls

- Raw material analysis

Product formulation

Well-controlled formulation parameters – **water activity (a_w), % moisture, pH, and antimicrobial preservation technology** – work in concert to create undesirable conditions for spoilage organism growth in pet treats and ingredients.

Water Activity and Microbial Growth



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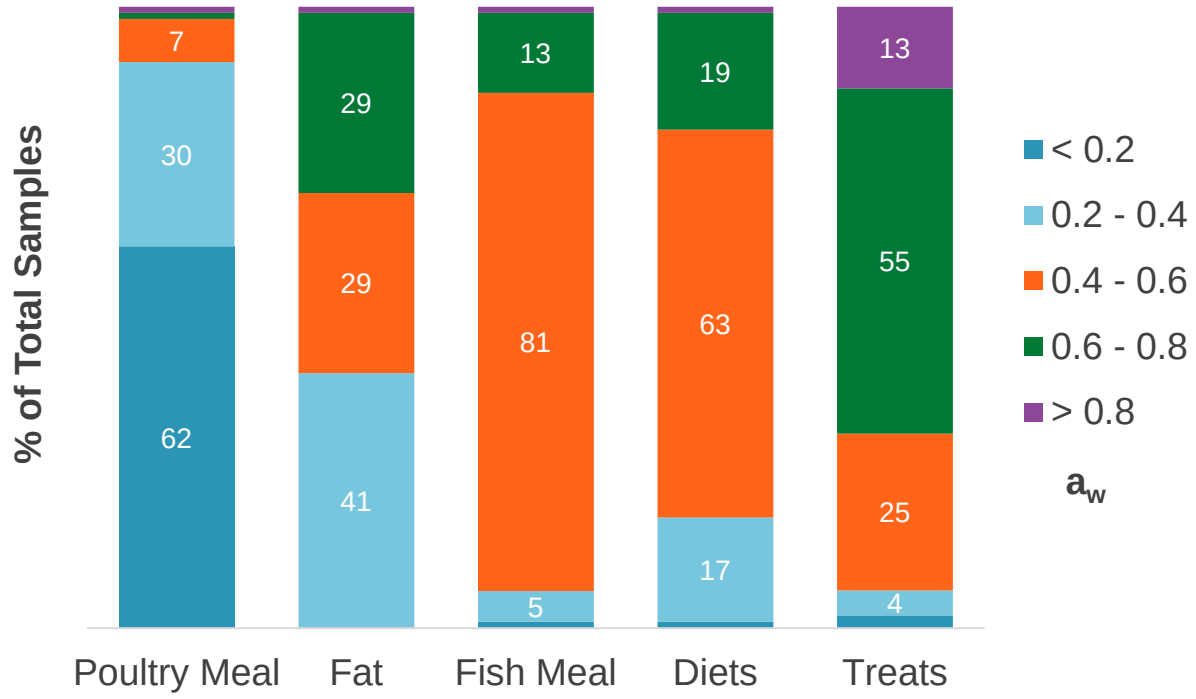
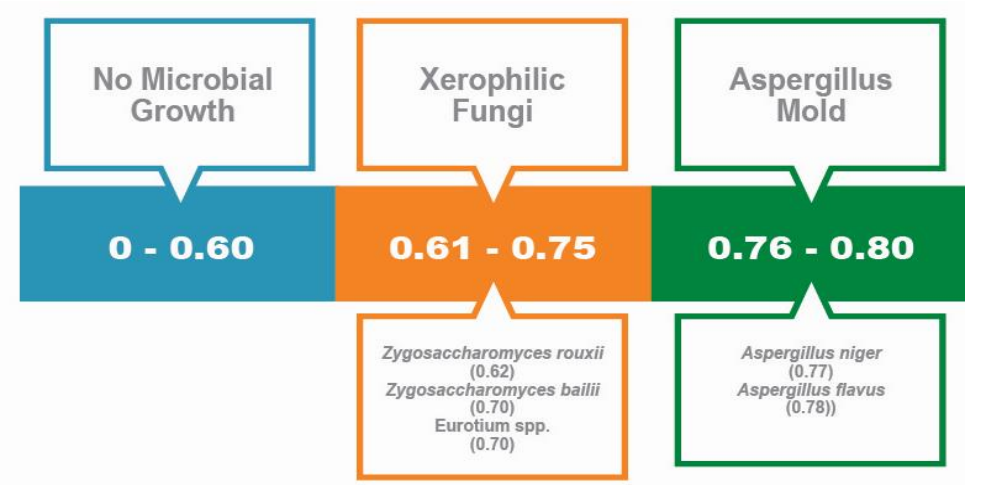
Lenovich, L.M. 1987. Survival and death of microorganisms as influenced by water activity. In: Rockland LB, Beuchat L (eds) Water activity: theory and applications to food. Marcel Dekker, Inc., New York, pp 119–136 Scholar

Water activity (a_w)

Water Activity and Microbial Growth





Risk Management Process Control:
Product formulation

- **Water activity (a_w) and % Moisture**
- pH
- Antimicrobial technology

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Technical Services and Lab Analysis

Services and Lab Analysis

Active Ingredient Recovery

- Application and blending confirmation

pH, Water Activity, Proximates (fat, protein, moisture)

- Product characterization

Microbial Enumerations

- Microbial load and risk determination

Application Research

- Processing, handling, ingredient compatibility, application point, dosage optimization

Treatment Efficacy Evaluations

Shelf-life

- Evaluates real-time interactions between a_w, moisture, pH, packaging, etc. at ambient temperature. Samples monitored daily for visual mold growth.

Mold Challenge

- Evaluates comparative efficacy between samples in presence of added humidity, optimized to accelerate mold growth (28°C, 86-90% Relative Humidity)
- Treatments may be *further* challenged by mold inoculum application, and added humidity

Microbial Spoilage and Application Analyses

- Water activity (a_w), pH, % moisture
- Incoming treat mold and yeast counts
- Acid recovery treatment quantification – requires untreated control

Challenge Model Learnings

- Assessment of manufacturing critical control points
- Validate lethality of pathogens through processing
- Evaluate effectiveness of preservatives
- Validate new or modified product formulation
- Appraise shelf-life risks

Challenge testing can be a powerful tool to evaluate effectiveness of thermal exposure, pressure processing, and food formulations to significantly reduce food safety risks

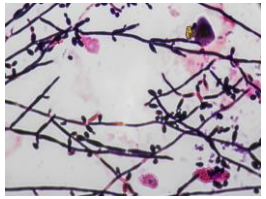
Growth Inhibition Food Spoilage Challenge

	Mold Challenge	Yeast Challenge
Challenge Organism	*Aspergillus ruber* at 10^6 cfu / mL	*Zygosaccharomyces bailii* at 10^5 cfu / g
Temperature Stress	Elevated (27°C ± 2°) or Ambient	Elevated (27°C ± 2°)
Moisture Stress	85% RH or Ambient	Ambient
Timeline	30 days	10 days

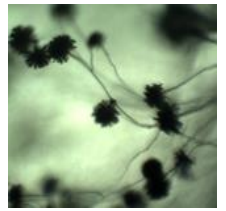
Spoilage Challenge in Pet Treat Model

How do natural preservatives perform against mold and yeast spoilage in pet treats?

Semi-Moist Treat
Model
a_w= 0.86
Moisture = 26%



Semi-Moist Treats + Solution



Xerophilic, extreme acid resistant yeast *Zygosaccharomyces bailii*

or

Xerophilic mold, pet treat isolate *Eurotium rubrum*

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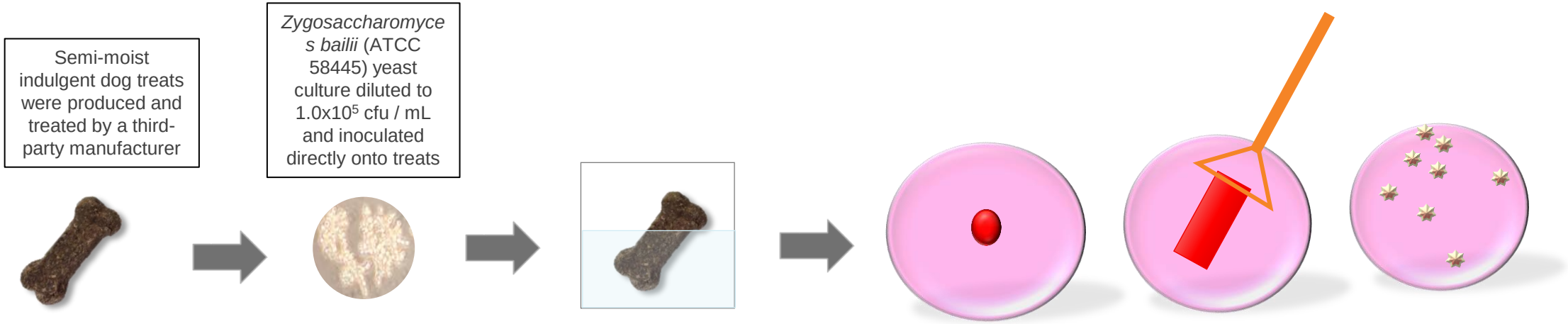
Yeast Method

Pet food spoilage model designed to permit growth of a low-moisture, acid-resistant, yeast strain

Allows investigation into yeast behavior and survival under specified conditions designed to model food product experiencing a worst-case scenario high yeast contamination event

Semi-moist indulgent dog treats were produced and treated by a third-party manufacturer

Zygosaccharomyces bailii (ATCC 58445) yeast culture diluted to $1.0x10^5$ cfu / mL and inoculated directly onto treats



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Mold Method

Pet food spoilage model designed to permit growth of a low-moisture mold strain isolated from commercial treats

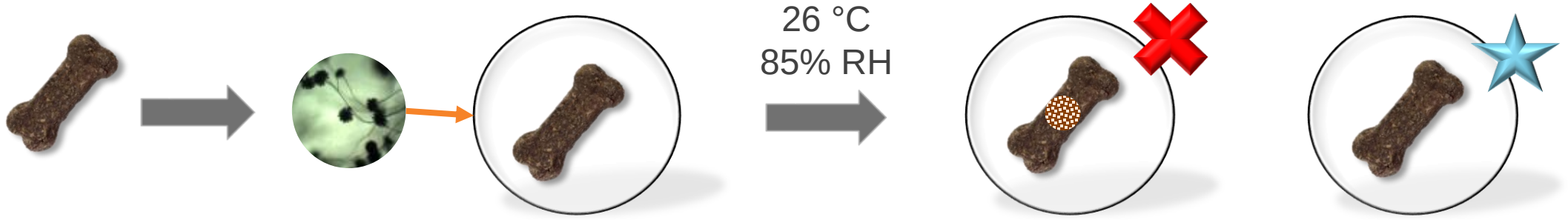
Allows investigation into mold behavior and survival under specified conditions designed to model food product experiencing a worst-case scenario high mold contamination event

Semi-moist indulgent dog treats were produced and treated by a third-party manufacturer

Aspergillus ruber mold culture diluted to 1.0×10^6 cfu / g and inoculated directly onto prepared treats

26 °C
85% RH

Treats were monitored for mold growth at 10-20X magnification in a climate-controlled chamber for up to 30 days



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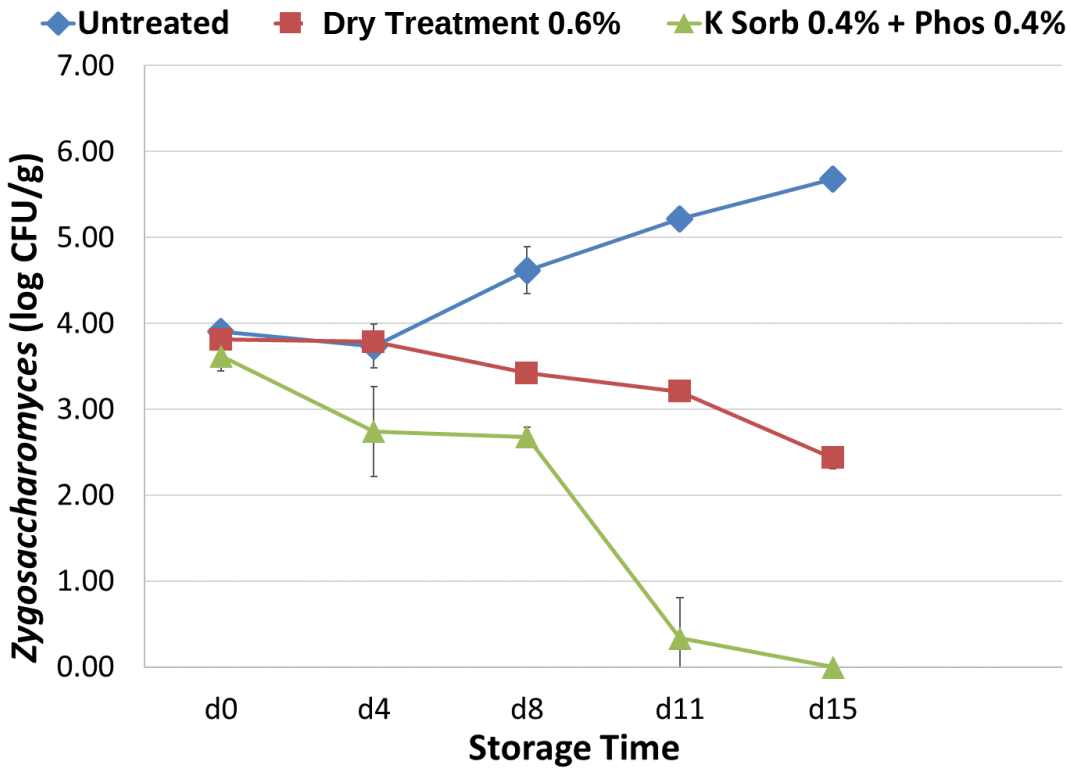
Yeast Growth in Treats



- Treat model sufficient to allow yeast growth (a_w 0.86)
- Yeast growth was significantly inhibited by natural and synthetic preservatives

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Data Source: Kemin-SD-17-00100

Medium Chain Fatty Acid

Common Approaches for Microbial Growth

	Microbial Target		
	Mold	*Yeast*	*Bacteria*
Acetic Acid and Buffered Vinegar	☑	☑	☑
Ascorbic Acid	-	-	☑
Citric Acid	☑	☑	☑
Lactic Acid & Lactates	-	-	☑
Phosphoric Acid	☑	☑	☑
Propionic Acids & Propionates	☑	-	☑
Sorbic Acid & Potassium Sorbate	☑	☑	☑
Phosphoric Acid	☑	☑	☑
Medium Chain Fatty Acids	☑	☑	☑

Organic Acid for Risk Management

Risk Management Process Control:

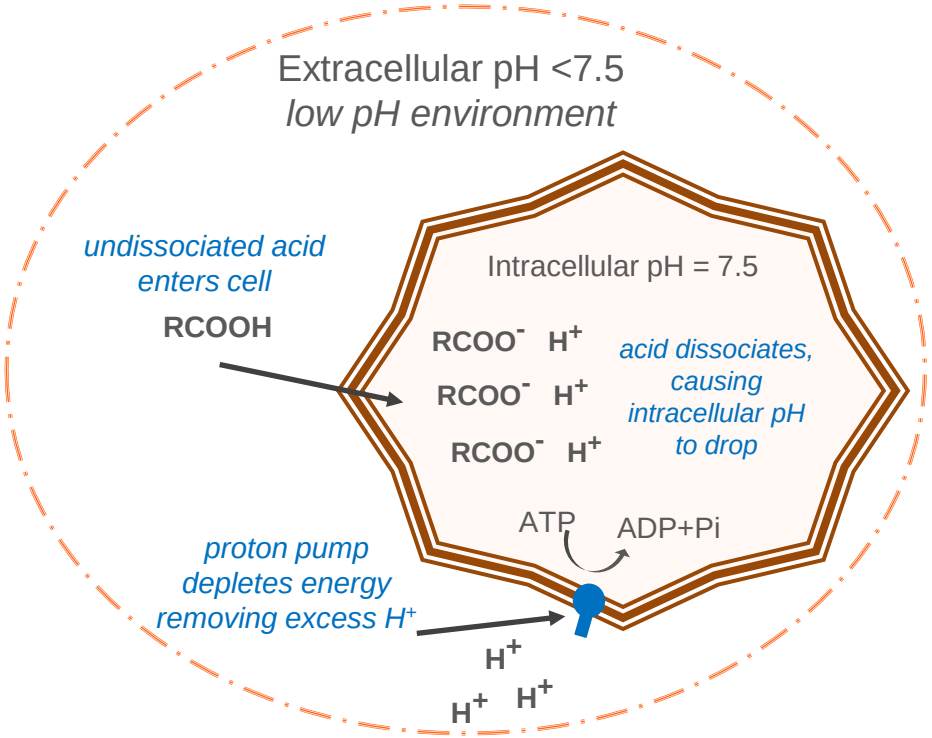
Product formulation

- Water activity (a_w) and % Moisture
- pH
- Antimicrobial technology

	pH		
Undissociated (%)	Propionic	Lactic	Acetic
99	2.87	1.83	2.76
95	3.59	2.55	3.48
90	3.92	2.88	3.81
80	4.27	3.23	4.16
70	4.50	3.46	4.39
60	4.69	3.65	4.58
50%	**4.87**	**3.83**	**4.76**
40	5.05	4.01	4.94
30	5.24	4.20	5.13
20	5.47	4.43	5.36
10	5.82	4.78	5.71
1.0	6.87	5.83	6.76
0.5	7.17	6.13	7.06

Organic Acid Mode of Action

- Undissociated acid (neutral charge) enters cell
- Acid dissociates inside cell, due to higher pH
- Charged dissociated acid cannot escape cell
- Accumulation acidifies cell and depletes energy



Normal cell physiology is disrupted by the combination of intracellular pH drop and cellular energy depletion

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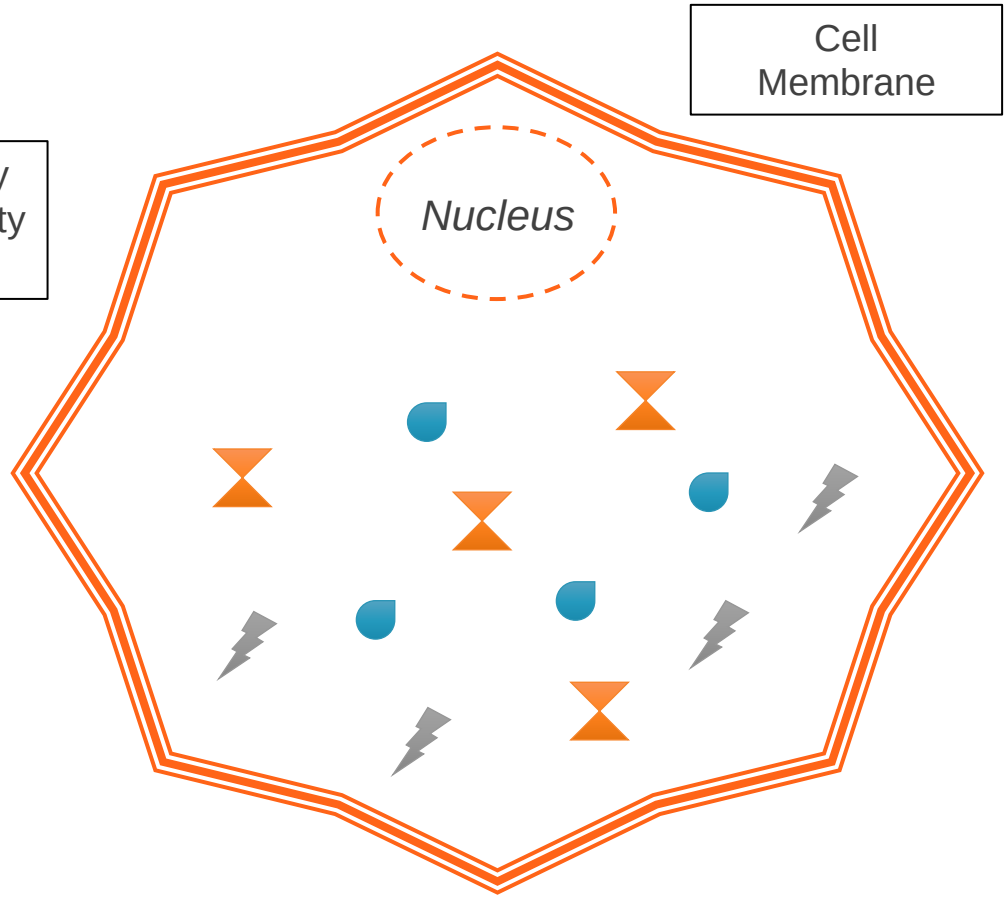
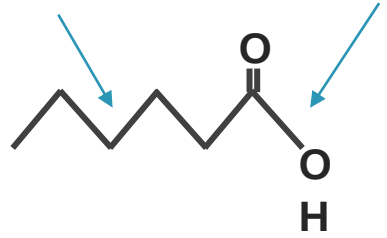
Membrane Disruption Mode of Action

Cellular leakage by Medium Chain Fatty Acid



Lipophilic carbon chain



Vital components of cellular matrix:

- ATP / ADP – energy
- DNA / RNA – protein synthesis
- Proteins – Cell functionality

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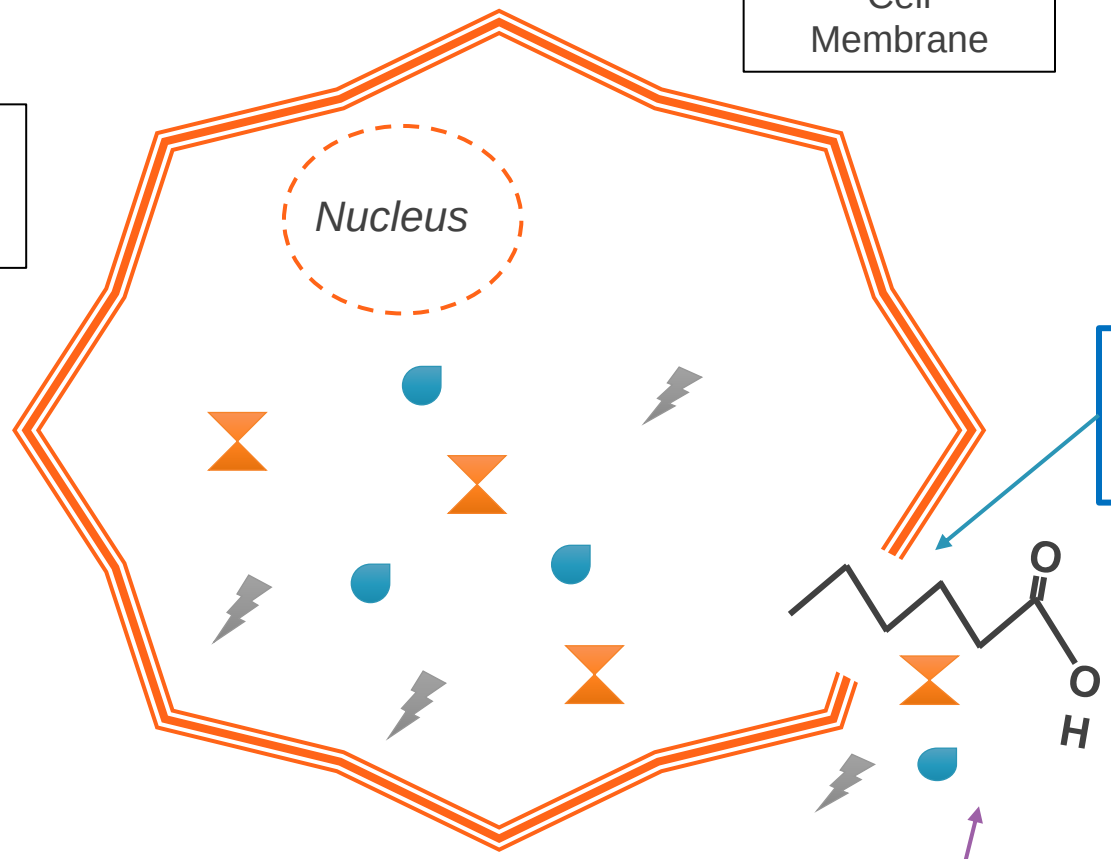
Membrane Disruption Mode of Action

Cell Membrane

Cellular leakage by Medium Chain Fatty Acid



Lipophilic carbon chain disrupts cell membrane causing leakage

***Result**: Vital cellular components spill out of cellular matrix*
→ cell death

- ATP / ADP – energy
- DNA / RNA – protein synthesis
- Proteins – Cell functionality

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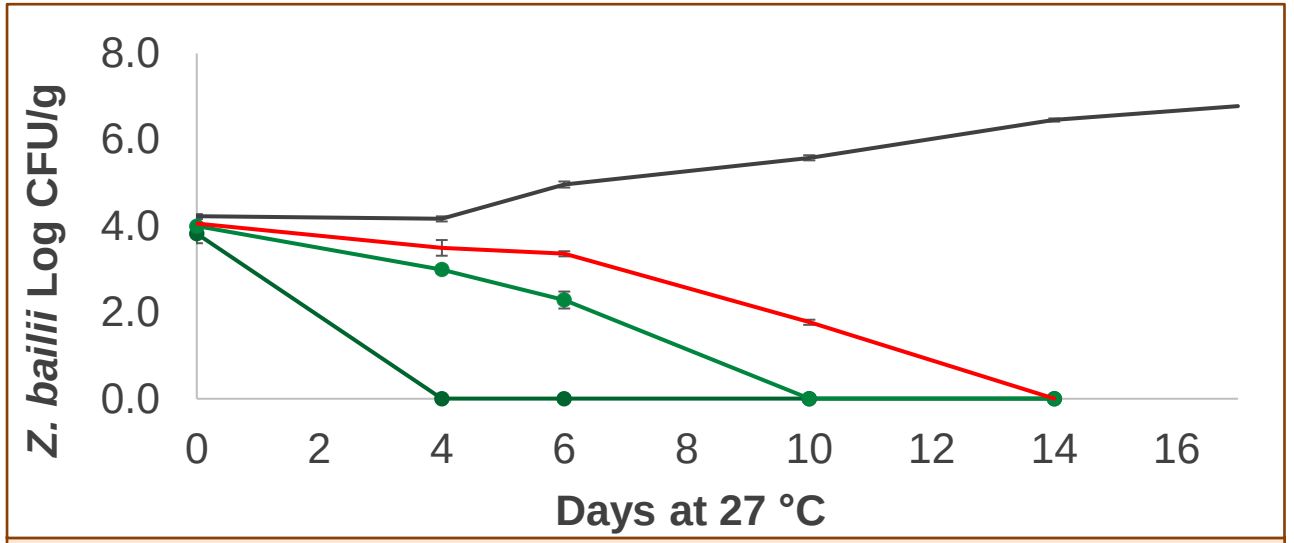
Yeast Control: MCFA

Inhibition in semi-moist indulgent dog treat challenged with *Zygosaccharomyces bailii* yeast

Yeasts Grow in Low Moisture Conditions



Treatment	pH	a_w	% Moisture
MCFA (0.6%) + Phos Acid (0.6%)	4.80	0.878	33.08
MCFA (0.4%) + Phos Acid (0.6%)	4.93	0.862	32.23
Cultured Whey (1.0%) + Phos Acid (0.6%)	5.00	0.853	29.48
Untreated + Phos Acid (0.6%)	5.19	0.853	31.12

Error bars represent standard deviation of mean growth (n=1, triplicate plates)

MCFA Liquid inhibits growth of resilient yeast strain known to spoil low water activity petfoods

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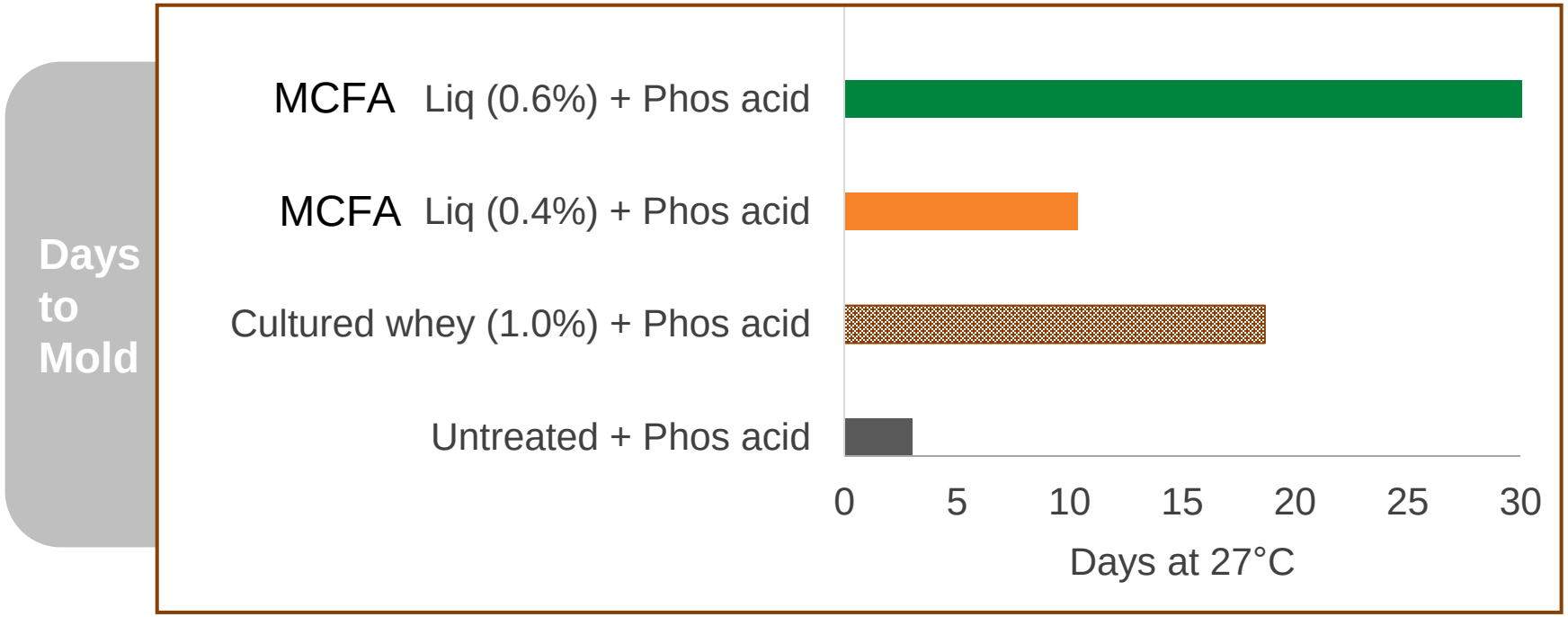
Data Source: Kemin-SD-2123851

Mold Control: MCFA

Inhibition in a semi-moist indulgent dog treat challenged with *Aspergillus ruber* mold



MCFA Liquid inhibits growth of hardy mold strain that can grow and reproduce with very little available water

Error bars represent standard deviation of mean days-to-mold (n=1, triplicate inoculation)

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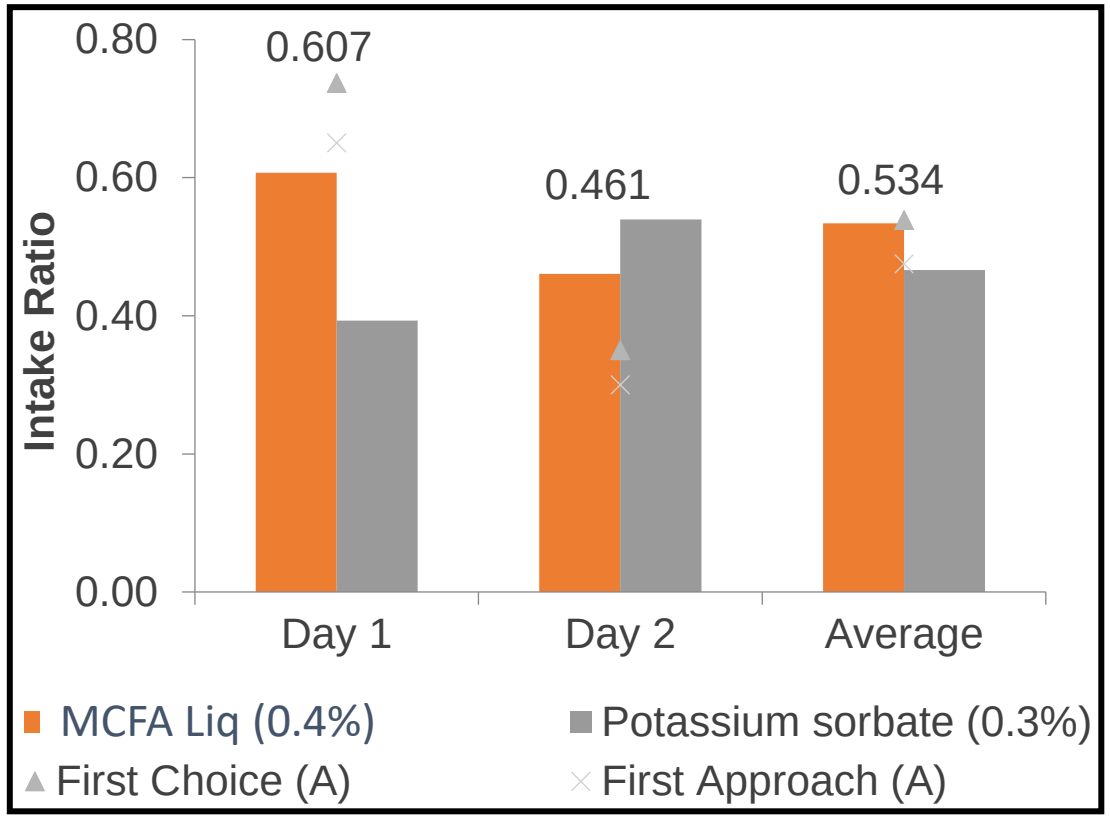
Data Source: Kemin-SD-2123851

Palatability: MCFA

- Palatability trials were conducted with dogs from Summit Ridge Farms (Susquehanna, PA, USA)
- Goal: evaluate palatability of MCFA Liq (0.4%) against synthetic control Potassium sorbate (0.3%)



MCFA Liquid is effective at a low dose, with Palatability accepted up to 0.4%

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Data Source: Kemin-TD-21-7001 and SD-2123851

Natural Spoilage Control in Pet Treats and Ingredients

Application rate

MCFA: 0.2% – 0.4%

When a microbial hurdle technology is applied to pet foods, the actual inclusion rate will depend upon formulation, storage conditions, and desired storage time of the pet food product.

Natural Pet Treat Preservation

MCFA Liquid provides natural shelf-life extension without acidification, for a versatile microbial hurdle option featuring pantry-friendly labeling.

MICROBIAL CONTROL

NATURAL SOLUTIONS

LABEL FRIENDLY

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