

#### Modernizing the Ways of Traditional Smoking: Application of Liquid and Dry Smoke in Meat, Poultry and Pet Foods

Organized by: IAFP's Meat and Poultry Quality and Safety PDG and Animal and Pet Food Safety PDG

Moderator: Saurabh Kumar, Kerry

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## Modernizing the Ways of Smoking: Application of Liquid and Dry Smoke in Meat, Poultry and Pet Foods



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Title	Speakers
History, Uses, and Regulatory Status of Liquid Smoke for Food Applications	Joshua B. Gurtler, USDA
Multifunctional Smoke Systems for Antimicrobial and Antioxidant efficacy in Meat / Poultry Application	Surabhi Wason, Kerry Ingredients
Liquid Smoke as a Clean-Label Solution for Pet food Applications	Aiswariya Deliephan, Kraft Heinz

## Overview of the History, Uses, and Regulatory Status of Liquid Smoke for Food Applications

Joshua Gurtler USDA, Agricultural Research Service Microbial and Chemical Food Safety Research Unit Eastern Regional Research Center, Wyndmoor, PA





USDA-ARS, ERRC Bio-Energy Program Sustainable Biofuels and Co-products Research Unit Fast Pyrolysis Reactors







#### Fast Pyrolysis Thermo-Chemical Conversion





#### History of Traditionally Smoking Meats



#### Colonial Smokehouses in Williamsburg, VA





USDA

#### Colonial Smokehouses in Williamsburg, VA





## *History* of Traditionally Smoking Meats

- Used for centuries/millennia in cultures around the world
- Involves heating wood/sawdust or other organic feedstocks (e.g., nut shells, coconut hulls, rice husks, etc.) at temperatures and/or oxygenlimiting conditions to promote smoldering and prevent burning
- Hardwoods are favored (e.g., mesquite, maple, oak, hickory, cherry, apple, beech, pecan, etc.), while some softwoods are acceptable
- Hypoxoic fast pyrolysis being studied for its production (>100 publications)





## *Benefits* of Traditionally Smoking Meats





## *Benefits* of Traditionally Smoking Meats

- Delays microbial spoilage and pathogen growth by the production of antimicrobials
- Delays oxidative spoilage by the production of antioxidants
- ➤Enhances color
- ➤Enhances aroma
- ➤Enhances flavor
- Enhances texture and promotes peeling on some meat products
- ➢ Dries/lowers a<sub>w</sub>







## *Challenges* With Traditionally Smoking Meats





## *Challenges* With Traditionally Smoking Meats

- Natural meat smoking is not economically or logistically feasible in many locations and for many (especially small) companies
- Important employee safety/health considerations during production
- Traditional smoking imparts some metabolically toxic and potentially carcinogenic chemical byproducts to foods (e.g., PAHs [Polycyclic aromatic hydrocarbons])

Natural smoke extract can contain >60 different PAHs, although formation is minimized at < 400°C</p>









#### Liquid Smoke, Smoke Condensates and Smoke Extracts aka Wood Vinegar or Pyroligneous Acid







USDA

Generated by capturing natural smoke condensates from heating wood or other organic feedstocks (e.g., rice husks)

➢Originally known as "wood vinegar"



- $\succ$ Generated by capturing natural smoke condensates from heating wood or other organic feedstocks (e.g., rice husks)
- ➢Originally known as "wood vinegar"
- Roman naturalist, Pliny the Elder, recorded its use as a superior embalming agent (ca. 50 A.D.)









Production from charcoal first published by German Alchemist Johann Rudolph Glauber (1658)





➢Wood vinegar first called pyroligneous acid in 1788

- Popularized and first marketed in the U.S. by Missouri pharmacist Ernest H. Wright in 1895 – used by farmers curing meats
- Historically applied to meat & poultry, fish & seafood, and nonmeat foods (e.g., nuts, cheese, tofu, beans, pet food, etc.)
- ➢ 2023 global market value of ca. \$93 million expected to reach \$245 million by 2033

https://www.futuremarketinsights.com/reports/liquid-smoke-market



#### *Benefits* of Industrial Liquid Smoke Application





https://en.wikipedia.org/wiki/Liquid\_smoke



# *Benefits* of Industrial Liquid Smoke Application

- Some benefits similar to traditional smoking (e.g., enhances color, aroma, and flavor, & includes antioxidants + antimicrobials to prevent microbial and oxidative spoilage)
- Toxic chemical compounds removed by refining condensates with phase separation and filtration (e.g., PAHs)





https://en.wikipedia.org/wiki/Liquid\_smoke



# *Benefits* of Industrial Liquid Smoke Application

- Reduces harmful smoke emissions in the vicinity during application (especially urban areas)
- Reduces other employee safety/health hazards
- Reduced labor costs for food application and cleanup
- Increased throughput





https://en.wikipedia.org/wiki/Liquid\_smoke







https://en.wikipedia.org/wiki/Liquid\_smoke









https://en.wikipedia.org/wiki/Liquid smoke



https://www.youtube.com/watch?reload=9&app =desktop&v=YJUGzk03qeM





https://en.wikipedia.org/wiki/Liquid\_smoke



- ➢ Research done in 1960s and 1970s refined modern processing
- ➢ Produced by batch or continuous feed, typically at 450-500°C
- ➢ Reactors include rotary ovens, heated augers, and fluidized beds
- Condenses into an aqueous phase and an oil phase, collected by water baths, scrubbers or shell and tube heat exchangers
- ➤Aged to increase sensory qualities
- Filtration and purification removes unwanted and harmful compounds (e.g., waxes, resins, terpenes, metals, PAHs, etc.)





https://en.wikipedia.org/wiki/Liquid\_smoke



### *Chemical Composition* of Smoke Condensates and Extracts





https://en.wikipedia.org/wiki/Liquid\_smoke



### *Chemical Composition* of Smoke Condensates and Extracts

- Important components include water, organic acids (e.g., acetic, propionic), carbonyls, and phenols (with other compounds, e.g. furans and furfurals), many impart tartness
- Condensate contain ca. 400 organic compounds, including ca. 85 phenols, 110 aldehydes and ketones, 65 carboxylic acids, 20 aliphatic hydrocarbons, 80 aromatic hydrocarbons, as well as alcohols, esters, etc.





https://en.wikipedia.org/wiki/Liquid\_smoke



## *Chemical Composition* of Smoke Condensates and Extracts

- Wood to generate liquid smoke composed of cellulose, hemicellulose, and lignins
- Cellulose & hemicellulose (pyrolyze at 180-350°C) generate organic acids & carbonyls (i.e., aldehydes & ketones) - impart sweetness, color, and antimicrobials
- Lignins (pyrolyze at 300-500°C) generate phenols - impart smoky flavors, aroma, antimicrobials, and antioxidants





https://en.wikipedia.org/wiki/Liquid\_smoke



*Chemical, Flavor & Aroma Profiles*, and *Functionality* Affected by Various Factors During Pyrolysis





https://en.wikipedia.org/wiki/Liquid\_smoke



*Chemical, Flavor & Aroma Profiles*, and *Functionality* Affected by Various Factors During Pyrolysis

- ➤Type of wood used
- Moisture content
- ➢Particle size
- ➤Temperature
- $>O_2$  concentration
- ➤Vapor residence time





https://en.wikipedia.org/wiki/Liquid\_smoke



#### Chemical, Flavor & Aroma Profiles, and Functionality Affected by Various Factors During Pyrolysis

Table 2. Organoleptic compounds found in LSC [34, 42].

Compound	Structure	Flavour
a-terpineol	H <sub>3</sub> C OH H <sub>3</sub> C CH <sub>3</sub>	<mark>sweet, floral, lilac</mark> flavour
3-hexenylbutanoate		wine-like, green, cognac or brandy, slightly buttery flavour
4-methylguajacol		spicy, aromatic, sweet, smoky, <mark>caramel, vanilla-like</mark>
linalool	H <sub>3</sub> C H <sub>2</sub> C H <sub>2</sub> C H <sub>2</sub> C H <sub>3</sub> C H <sub>2</sub> C H <sub>3</sub> C	<mark>light lavender</mark> flavour

Simon, et al., 2005. Composition and analysis of liquid smoke flavouring primary products. *Journal of Separation Science*. 28:871-882



#### Chemical, Flavor & Aroma Profiles, and Functionality Affected by Various Factors During Pyrolysis

terpinen-4-ol	H <sub>3</sub> C H <sub>3</sub> C H <sub>0</sub> C H <sub>3</sub> C H <sub>3</sub> C	pepper, woody, earthy, musty
<i>endo</i> -borneol	H <sub>3</sub> C CH <sub>3</sub> CH <sub>3</sub> OH	camphoraceous, earthy, <mark>pine</mark>
2H-1-benzopyran-2-one		fragrant, resembling vanilla beans, burning taste
guaiacol	О-	phenolic, <mark>spicy,</mark> peppery hot, sweet, dry, smoky
4-methoxyguajacol	оон	phenolic, smoky, burnt, <mark>Whiskey,</mark> dry, peppery hot
<i>i</i> -eugenol	О-	sweet fruity, mildly smoky, dry, peppery hot



Simon, et al., 2005. Composition and analysis of liquid smoke flavouring primary products. *Journal of Separation Science*. 28:871-882

### *Commercially Marketed* Condensed Liquid Smoke Preparations





https://en.wikipedia.org/wiki/Liquid\_smoke



## *Commercially Marketed* Condensed Liquid Smoke Preparations

- 1. Concentrated liquids for atomizing, or smoke regeneration in smoking/cooking chambers
- 2. Extracts added to meat by injection or mixing
- 3. Water-miscible solutions for direct liquid surface applications
- 4. Powders added to carriers to provide smoke flavor
- 5. Condensates to be directly added to sauces for meat application





https://en.wikipedia.org/wiki/Liquid\_smoke






https://en.wikipedia.org/wiki/Liquid\_smoke



➢ Recirculating showers or drenches



Rozum, J. 2014. Liquid Smoke (Smoke Condensate) Application. *Encyclopedia of Meat Sciences.* Vol. 3. pp. 315-320



- ➢ Recirculating showers or drenches
- Smoke regeneration (vaporization or atomization) - Recommended application rates of 1.5-3.0 kg condensate/ton meat



Rozum, J. 2014. Liquid Smoke (Smoke Condensate) Application. *Encyclopedia of Meat Sciences.* Vol. 3. pp. 315-320



- ➢ Recirculating showers or drenches
- Smoke regeneration (vaporization or atomization) - Recommended application rates of 1.5-3.0 kg condensate/ton meat
- Incorporating into ingredients, breading, batters, other coatings
- ≻Injecting
- ➢Spraying, dipping, brining
- Impregnated into meat casings, stuffed and cooked



Rozum, J. 2014. Liquid Smoke (Smoke Condensate) Application. *Encyclopedia of Meat Sciences.* Vol. 3. pp. 315-320



## Regulatory Status of Liquid Smoke Application for Meats and Poultry





https://en.wikipedia.org/wiki/Liquid\_smoke



Regulatory Status of Liquid Smoke Application for Meats and Poultry

> Classified as a Generally Recognized as Safe (GRAS) compound by the FDA





https://en.wikipedia.org/wiki/Liquid\_smoke









USDA Agricultural Research Service

## Regulatory Status of Liquid Smoke Application for Meats and Poultry





## Regulatory Status of Liquid Smoke Application for Meats and Poultry

"... the labeling of natural smoke flavorings is covered by <u>9 Code of Federal Regulations 317.2</u> (j) (3) and <u>381.119 (a)</u> and by <u>Policy Memo 117,</u> <u>"Smoke Flavoring</u>." Natural smoke flavoring may not be listed as "natural flavor" or "flavor" in the ingredients statement. It may be declared as "natural smoke flavoring" or "smoke flavoring."

https://ask.usda.gov/s/article/askFSIS-Public-Q-A-sf





https://en.wikipedia.org/wiki/Liquid\_smoke



### Regulatory Status of Liquid Smoke Application for Meats and Poultry 9 CFR 317.2 (j) (3)

"When an artificial smoke flavoring or a smoke flavoring is added as an ingredient in the formula of a meat food product, as permitted in part 318 of this subchapter, there shall appear on the label, in prominent letters and contiguous to the name of the product, a statement such as "Artificial Smoke Flavoring Added" or "Smoke Flavoring Added," as may be applicable, and the ingredient statement shall identify any artificial smoke flavoring or smoke flavoring so added as an ingredient in the formula of the meat food product."

https://www.ecfr.gov/current/title-9/chapter-III/subchapter-A/part-317/subpart-A/section-317.2





https://en.wikipedia.org/wiki/Liquid\_smoke



Regulatory Status of Liquid Smoke Application for Meats and Poultry 9 CFR 317.2 (j) (3) However. . .





https://en.wikipedia.org/wiki/Liquid\_smoke



#### Regulatory Status of Liquid Smoke Application for Meats and Poultry 9 CFR 317.2 (j) (3) However. . .

"Meat or poultry products which have been exposed to natural liquid smoke flavor which has been <u>transformed into a true gaseous state by the</u> <u>application of heat or transformed into vapor by</u> <u>mechanical means</u>, e.g., atomization, may be labeled "<u>Smoked</u>.""

USDA, FSIS. 2024. Food Standards and Labeling Policy Book. https://www.fsis.usda.gov/sites/default/files/import/Labeling-Policy-Book.pdf



Rozum, J. 2014. Liquid Smoke (Smoke Condensate) Application. *Encyclopedia of Meat Sciences.* Vol. 3. pp. 315-320



#### Regulatory Status of Liquid Smoke Application for Fish and Fishery Products 21 CFR 123.3 (s)





https://en.wikipedia.org/wiki/Liquid\_smoke



## Regulatory Status of Liquid Smoke Application for Fish and Fishery Products 21 CFR 123.3 (s)

"Smoked or <u>smoke-flavored</u> fishery products means the finished food prepared by: (1) Treating fish with salt (sodium chloride), and (2) Subjecting it to the direct action of smoke from burning wood, sawdust, or similar material and/or <u>imparting to it the flavor of smoke</u> by a means such as <u>immersing it in a solution of wood smoke</u>."

https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-123





https://en.wikipedia.org/wiki/Liquid\_smoke



#### Thank You for Your Attention

USDA, Agricultural Research Service Eastern Regional Research Center • Wyndmoor, PA











Multifunctional Smoke Systems for Antimicrobial and Antioxidant efficacy in Food Application

**Revolutionizing Food Preservation With Innovative Clean Label Smoke Technology** 

# Smoke solutions for meat and poultry preservation

Why smoke?

Issues in safety and quality of meat?

How smoke can preserve meat?

Delaying spoilage: Fresh Poultry

Shelf-life extension and *Listeria* control: Frankfurters

Salmonella control in fresh poultry

Sensory validation

Other applications - Beverages



## Bacteria impacting the meat safety and stability

#### **Common Defects in Meat Products & Causal Bacteria**

Defects	Meat product	Causative Bacteria
Slime	Meats	Pseudomonas, Lactobacillus, Enterococcus, Weissella, Brochothrix
H <sub>2</sub> O <sub>2</sub> Greening	Meats	Weissella, Leuconostoc, Enterococcus, Lactobacillus
H <sub>2</sub> S Greenin	Vacuum Packaged Meats	Shewanella
H <sub>2</sub> S Production	Cured Meats	Vibrio, Enterobacteriaceae
Sulfide Odor	Vacuum Packaged Meats	Clostridium, Hafnia
Cabbage Odor	Bacon	Providencia
Putrefaction	Ham	Enterobacteriaceae, Proteus
Bone taint	Whole Meats	Clostridium, Enterococcus
Souring	Ham	Lactic acid bacteria, <i>Enterococcus,</i> <i>Micrococcus, Bacillus, Clostridium</i>

**Microbial risk for meat safety** 

Risk	Causative Bacteria	
INFECTION	Salmonella, Campylobacter jejuni, Listeria monocytogenes	
Production of TOXINS	Staphylococcus aureus, Bacillus cereus, Clostridium botulinum	
	© Kerry	

Nychas et al. (2008)

#### How to maintain food stability and food safety



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# Smoking of poultry, fish and red meat has been increasing in popularity

#### **Benefits of Liquid smoke**

- 1. Application of liquid smoke requires less time
- 2. Ease of application
- Use of liquid smoke allow the processor to control the concentration of smoke being applied – good reproducibility of desired characteristics obtained in the final product



\*https://www.marketreportsworld.com/enquiry/request-sample/21011034

## The Power of Smoke /



One package preservative solution

# In-vitro studies



## Antimicrobial efficacy in-vitro of liquid and dry smoke products

#### MIC % (Minimum inhibitory concentration) at pH= 6.0 and Temp= 30 °C



#### Conclusion

Antimicrobial efficacy in-vitro of Smoke A> Smoke D> Smoke B > Smoke C

Smoke concentration (%)



# Application studies

#### Antimicrobial efficacy of liquid smoke against spoilage and pathogenic bacteria in meat application



L. monocytogenes

Zesti Smoke @ 2.5% LS -2 log CFU/mL reduction;
5% LS -listericidal after 6 weeks;
10% LS -listericidal after 4 weeks (Morey et al., 2012)

Zesti B 100% sprayed - Undetectable 1 log -after 1 week; 2 log -after 4 weeks; 3 log -slowly increased to 3.8 logs over 10 weeks Control -increased by 7 logs.

Frankfurters

**CharSol-10 100% dipped -** 1 log reduction after 72 h (Messina et al., 1988)

Ground pork bellies

> **Charsol Supreme @1.25%** - 1.7 log reduction after 3 h (Taormina and Bartholome, 2005)

**Staphylococcus** 

aureus

# Antimicrobial efficacy of liquid smoke against spoilage and pathogenic bacteria in seafoods application



Trout

C10 Staphylococcus aureus
25% No reduction after 5 days at 25%.
50% Below detection after 5 days
75% Below detection after 3 days
100% Below detection after 3 day
(Paranjpye et al., 2004)

Salmon

AM-3, AM-10 @0.9% 2 log CFU/g reduction *Listeria innocua* after 2 weeks (Montazeri, Himelbloom et al., 2013)

**CharSol Supreme 60%,** 15 s dip 3 log reduction of *Listeria innocua* (Vitt et al., 2001)

Smoke fractions 100% dip, 1 min
L. monocytogenes L1 and L2 - below detection limit immediately;
S - below detection limit by 21 days;
L3 - no inhibitory affects (Suñen et al., 2003)

## Additive Combinations for Delaying Spoilage in Fresh Poultry

Market **Dynamic** 

Shelf life without preservation is very short, needing to be ground onsite at the retailer and often creating large quantities of retail and consumer waste.





**Before** 

No preservative

#### After

- Vinegar
- Natural (Smoke) Flavour

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#### Refrigerated Fresh Poultry treated with Buffered Vinegar and Smoke had An Enhanced Shelf Life

Fresh Poultry Aerobic Plate Count at 4°C pH: 6.5 | Moisture: 75% | Salt: 0.5%



13 1 31 19 5

## The use of multifunctional ingredients to improve food safety

Clean label shelf-life extension solutions for meat applications



agrice Mit Con



Microbial and oxidative delay are possible with clean label ingredients





Extra shelf-life days can translate into positive environmental impact



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### Enhancing Sensory, Texture and Shelf Life of Hot Dogs



Hot dogs are affordable and delicious meat products, beloved around the world. They have a long shelf-life expectation from retailers and consumers alike.

Approach

Im



Hurdle approach: Combining organic acids with an ingredient with a different mode of action can extend shelf life through additive effects.

npact	Extra Shelf-Life Days with 1.5% Cloud 230 + 0.63% IsoAge DV100	Listeria	L. sakei
	Versus Negative Control (no preservatives)	>60 days	15 days
	Versus Market Solution (IsoAge DV100)	28 days	13 days



After

- Vinegar
- Natural Smoke Flavour



Microbes of concern: Pathogen (*Listeria* monocytogenes cocktail), Spoilage (*Lactobacillus* sakei)

**15 days+** Minimum additional shelf life versus no preservatives



#### Combined Efficacy of Vinegar and Smoke on Refrigerated Hot Dog Over Shelf Life

Hotdogs Listeria cocktail at 4°C pH: 6.7 | Moisture: 64% | Salt: 2.77%



**Conclusion:** Hot Dogs treated with IsoAge DV100 in combination with Cloud 230 have been found to have a longer shelf life - stored at 4°C.

Hotdogs L. sakei cocktail at 4°C pH: 6.7 | Moisture: 64% | Salt: 2.77%

## **Sensory Analysis**

Sensory analysis was conducted to determine the sensorial impact of the Cloud 230 at three usage levels on key frankfurter attributes.

#### Methods:

- Samples were blinded with three-digit codes and compared to reference (0.63% IsoAge DV100).
- Panelists used a 9-point relative to reference scale to determine how different and the directionality of that difference (more or less) for key attributes.

#### **Data Analysis:**

The data was collected using EyeQuestion version 5.07.15 and analyzed and charted using the Kerry Data Analysis Software.

## Addition of Cloud 230 enhances Umami and Bite





Day 30

(Blind) 0.63% IsoAge DV100
0.63% IsoAge DV100+ 1.0% Cloud 230



0.63% IsoAge DV100+ 0.5% Cloud 230
 0.63% IsoAge DV100+ 1.5% Cloud 230

#### © Kerry 2024 | 68

#### Salmonella Inhibition in Fresh Chicken

Market Dynamic

- Salmonella -10 large-scale recalls in the US in 5 years (Source: USDA FSIS 2019 – 2024).
- About 1.35 million cases of salmonellosis, with 26,500 hospitalizations and 420 deaths (Source: Centers for Disease Control and Prevention (CDC).

There are limited ingredients today that will protect food in the event of a *Salmonella* contamination.



Microbe of concern: Salmonella



# Evaluation of Smoke as an Intervention for Salmonella spp. in Ground Chicken



#### Challenge Study: Log Reduction of Salmonella in Various Fresh Poultry Formulations

Fresh poultry: Effect of Smoke A and Smoke B against Salmonella pH= 6.0; aw =0.962; Temp =4°C; MAP packaging (30% carbon dioxide and 70% nitrogen)



#### Conclusion

- 1. 0.3% Smoke B provided 0.6 log reduction maintained throughout storage.
- 2. 0.5% Smoke A achieved 0.3 log CFU/g reduction of Salmonella cocktail in ground poultry which remained stable throughout storage.
- 3. 0.6% Smoke B achieved 1.40 log CFU/g reduction of *Salmonella* cocktail in ground poultry which remained stable throughout storage. This treatment could impart smoky flavor and color so advantageous for applications such as smoked sausages.

### Sensory Validation of Various Ground Poultry Concepts Containing Smoke and Vinegar

Kerry Bench Review (KBR) system was used to evaluate the sensory characteristics by 17 panelists. **Attributes evaluated**: Acetic, Smoky, salty, sour, sweet, meaty, roasted, texture, appearance, and overall aftertaste.



#### Conclusion

- 1. Overall, there is an increased perception of smoky, roasted, acetic, and overall aftertaste with inclusion of 0.5% Smoke A and 0.3% Smoke B.
- 2. Metallic/Canned perception of samples with smoke were not significant different as compared to control.
- 3. 0.3% Smoke B imparted slightly bitter taste as compared to 0.5% Smoke A.
- 4. Addition of vinegar did not impact the sensory perception. Combination of smokes and vinegar at 0.49% can be used for ground chicken application.
### Antioxidant Analysis of smoke fractions (In vitro) via DPPH

### Comparison of Different Smokes with High Antioxidant Activity





Several smoke fractions were evaluated for radical scavenging capacity and found to have moderate to excellent antioxidant capabilities.



## Potential of Smoke in diverse food matrices



Yeast and Mold inhibition in Beverages, Supplements, Plant Based and Dairy

### Carbonated Beverage: Yeast Challenge Study

### Aim

To evaluate efficacy of Flavoset 5400L in carbonated beverage against Yeast.

### **Trial Conditions**

- Products prepared in commercial trial
- 100 mL aliquoted into crimp top vials with rubber stopper (3 samples/treatment)
- Treatments inoculated with yeast cocktail at 2-4 log CFU/mL, mixed gently to attain uniform dispersion of inoculum
- Samples stored at 25 °C throughout the study.

**Microbiological analysis** 

- Tested in Triplicate
- Yeast and Mold on PDA incubate at 25 °C for 4-7 days.

## Product Characteristics & Sensory

• pH, water activity (aw), colour

S.no.	Treatments	Smoke %
1	Untreated Control	-
2	Flavoset 5400L	0.75

S. No.	Strain name
1	Zygosaccharomyces bailii
2	Saccharomyces cerevisiae
3	Candida tropicalis
4	Dekkera bruxellensis
5	Brettanomyces naardensis

### **Carbonated Beverage - Antimicrobial Screening**





### **Product characteristics**

S. no.	Treatment	рΗ	Aw	Brix
1	T2:0.75% Flavoset 5400L	3.42	0.9390	2.38
2	T5: Control	3.52	0.9488	2.18

### Conclusion

- 1. T5 (no antimicrobial control) reached spoilage limit around day 6
- 2. Yeast levels in T2: 0.75% Flavoset 5400L below detection limit post day 3 timepoint and continued to be below detection limit for >72 days.

### Carbonated Beverage - Antimicrobial Screening



### **Product characteristics**

S. no.	Treatment	рΗ	Aw	Brix
1	T2:0.75% Flavoset 5400L	3.42	0.9390	2.38
2	T5: Control	3.52	0.9488	2.18

### Conclusion

- 1. T5 (no antimicrobial control) reached spoilage limit around day 10
- 2. LAB levels dropped below detection limit in T2, on day 3 and continued to be below detection limit for >72 days

### Smoke shows potential efficacy in a variety of applications



### Cloud Smokes key takeaways

- 1) Cloud smokes provide a broad-spectrum antimicrobial efficacy.
- 2) Clean label preservative solution for wide variety of food products.
- 3) Smoke based solutions were effective in inhibiting and controlling the growth of *Salmonella* in fresh poultry.
- 4) Smoke solutions did not impact the sensory perception.
- 5) Multifunctional Systems- Combined technologies / products are more effective to meet specific goals.

### **Cloud Smokes**

### Label Friendly

Natural smoke enables consumer-friendly flavor labelling.

### **Product Quality**

Best Before: DD MM YYYY

Smoke attributes inherently maintain quality over shelf life.

### **Sustainable**

Captures the best of the smoking process and removes and repurposes undesirable fractions.

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### IAFP WEBINAR

Modernizing the Ways of Traditional Smoking: Application of Liquid and Dry Smoke in Meat, Poultry and Pet foods

### APPLICATION OF LIQUID SMOKE IN THE MITIGATION OF MOLDS AND MITES IN A SEMI-MOIST PET FOOD MODEL

Aiswariya Deliephan, Ph.D.

Senior Scientist, Kraft Heinz

September 30, 2024

## Outline

- Introduction to intermediate moisture/semi-moist pet foods
- Spoilage & food safety issues
- Current challenges with conventional additives
- Liquid smoke: What we know/do not know
- Case studies:
  - Liquid smoke as an acaricide to control mite infestation in semi-moist pet food
  - Liquid smoke as an antimycotic to control mold spoilage in semi-moist pet food

## Introduction

- Semi-moist pet food
  - A popular **intermediate moisture food** in the pet food category
  - Current worldwide revenue of \$2.65 billion out of a total \$70 billion revenue from pet foods
  - Primarily contains meat and grain-based ingredients, fat, 20-30% moisture, with 0.76 a<sub>w</sub> Example: Chewy dog treats
- Spoilage & food safety issues in semi-moist pet food
  - Baked/formed (no kill step)
  - High moisture & a<sub>w</sub>; rich in proteins & fats
  - Very conducive for storage mold and mite infestation
    - *Tyrophagus putrescentiae* (cheese mite, ham mite or mold mite)
    - Aspergillus flavus, Fusarium graminearum (storage molds)
    - Mycotoxins like aflatoxin, zearalenone, DON
    - Wild-type mold (environmental/mixed molds)



Semi-moist pet food - Dog Treats



Mold infected pet food



Mite infested pet food

### Pet food industry challenges with conventional additives

Additive	Advantages	Challenges
Propylene glycol	<ul> <li>✓ Humectant (controls a<sub>w</sub>)</li> <li>✓ Used in dog food</li> <li>✓ Keeps mites away</li> </ul>	<ul> <li>Synthetic (confused with anti-freeze 'ethylene glycol')</li> <li>Toxic to cats (Heinz body formation in blood)</li> </ul>
Glycerin	<ul> <li>✓ Natural</li> <li>✓ Humectant (controls a<sub>w</sub>)</li> <li>✓ Used in dog &amp; cat foods</li> </ul>	× Favorable for mites
Potassium sorbate	<ul> <li>Mold inhibitor</li> </ul>	× Synthetic

**Solution:** A NATURAL, FOOD-SAFE additive with MULTI-functionality

Liquid smoke

## Liquid smoke

- Produced by condensing wood smoke created by pyrolysis of sawdust or wood chips followed by removal of carcinogenic hydrocarbons
- Contains carbonyls, phenols & organic acids in various proportions
- Natural, GRAS
- Color & flavor additive
- Antimicrobial



Liquid smoke



## Liquid smoke as an acaricide & antimycotic

What we know	What we do not know	
Wood smoke from different plants used to control 'bee mites' in apiaries <sup>1</sup> .	Can liquid smoke be effective against stored food mites?	
Liquid smoke (phenolic fraction) is effective against pathogenic bacteria like <i>Listeria</i> , <i>E.coli, Staphylococcus</i> in some meat products <sup>2,3</sup> .	What will be the effects of liquid smoke fractions against storage molds? How can it affect mold growth pattern & shelf life in a food substrate?	
Liquid smoke is effective against the mold Aspergillus niger in a nutrient broth assay <sup>4</sup> .		

## **Case Study 1:**

# Liquid smoke as an acaricide in semi-moist pet food

## Background

- *Tyrophagus putrescentiae* (cheese mite or mold mite)
  - Cosmopolitan stored-product mite species of the food industry including pet foods
  - Cause allergic asthma and rhinitis among food industrial workers
  - Cause allergy in dogs with atopic dermatitis; acute enteritis in humans on ingestion of mite-infested product
  - Vector for transmission of pathogens
- Isolated from pet food industry locations<sup>5</sup>, and sealed commercial dog food bags<sup>6</sup>
- Current mitigation strategy fumigation & pesticides (DEET); unsafe for the environment and human workers
- Liquid smoke can be a natural alternative







Mites under a microscope (used in this study)

### Liquid smoke fractions used in the study

SMOKE	NAME	DESCRIPTION
S1	P-1720	Buffered Low Phenol Smoke, Medium Carbonyl
S2	Cloud S-5	Buffered pH, Low Acid, Low Carbonyl, No Phenol
S3	Cloud S-C100	Carbonyl Preparation: High Carbonyl, Low Acid, Very Low Phenol
<b>S</b> 4	Black deli	Basic pH Smoke, Zero Carbonyls, Organic Acid Salts, Phenols
S5	Hickory OS 1473	Phenol Preparation: High Phenol, Low Acid, No Carbonyl
<b>S</b> 6	Code 10	Base Smoke: Organic Acid/Carbonyls/Phenols
S7	Code V	Organic Acid Preparation: Low pH, Medium Acid, Medium Carbonyl, Low Phenol
<b>S</b> 8	Cloud S-AC15	High Buffered Organic Acid + Medium Carbonyl Preparation

### **Experiment 1**: Mite reproduction & survivability assay

Semi-moist pet food



**Procedure:** 





9/27/12

Mites observed in the assay

Mean mite population growth at 7 and 14 days on semi-moist pet food treatments with inclusion of liquid smoke preparations P-1720, Cloud S-5 and Cloud S-C100 at 0.3% w/w, in comparison to the untreated (0% smoke) and mite rearing diet. The positive control (propylene glycol treatment) did not show mite population growth.



Each mean is based on *n* = 3 replications. Means among treatments across **day 7 and day 14** followed by different letters in **lower case** are significantly different (*P*<0.05, Tukey's test). Means among treatments within each time period of 7 or 14 days followed by different letters in upper case are significantly different (*P*<0.05, Tukey's test).

# Experiment 2: Mite orientation behavior assay (attraction/repellency)

### **Procedure:**

- Two-choice behavioral assays with mites in bio-assay arenas
- A 'treated' and a 'control' pet food cube placed in each of the circle **B** and **C**
- 20 adult mites released into middle circle A
- Arenas placed in darkness at 25°C and 70% RH
- Mites that oriented towards each of the pet food cubes within the circles counted after 1, 8 and 24 h
- Attraction or repulsion of mites quantified as Repellency Index (RI)

### $RI = (N_{c} - N_{t})/T \times 100$

where  $N_c = no.$  of mites on control;  $N_t = no.$  of mites on treated; T = total number of mites released (T=20)



Bioassay arena for two-choice behavioral assay of mites

Mean mite repellency indices (RI) for liquid smoke preparations at 100%, 50%, 25%, 10%, 5%, 1% and 0.3%, coated on semi-moist pet food at enumeration time points 1 h, 8 h, and 24 h. Positive control (propylene glycol treatment) showed 100% repellency. RI values which are "+" indicate repellency and "-" indicate attraction.



**Cloud S-5** 

Black deli

Code V



Hickory OS-1473











Concentration (%)

## Conclusions

- On average, at 24 h, P-1720 attracted mites the most (+20 to -50% RI), while Cloud SC-100 repelled them the most (-10 to +60% RI)
- Cloud SC-100, Code-10 and Cloud S-AC15 which contained high to medium carbonyl concentration may provide some repellency to retard mite infestation in stored semi-moist pet food

## Case Study 2:

## Liquid smoke as an antimycotic in semimoist pet food

## Background

- Aspergillus flavus & Fusarium graminearum
  - Predominant genera isolated from commercial pet foods<sup>7</sup>
  - Mold spores from environment contaminate packaged foods opened by the consumer and amplify during storage
  - Produce mycotoxins (aflatoxin, DON & zearalenone) toxic to humans & pets
  - Cooking does not reduce mycotoxin content
  - Pet fatalities & dog food recalls due to aflatoxin Sportmix<sup>™</sup> & Sunshine Mills, 2020
- Wild-type mold
  - Environmental/mixed mold
  - Relevance to the consumer; aesthetic quality
- Liquid smoke can be a natural intervention to mitigate mold growth in semi-moist pet food



Aspergillus flavus



Fusarium graminearum



Wild-type spoilage mold on pet food

### **Experiment 1: Mold challenge study with Aspergillus flavus**

### **Procedure:**

**Step 1:** Initial screening of smoke concentrations in nutrient broth assays (MIC & MFC)

Step 2: Evaluating in semi-moist pet food

1 mL of fungal inoculum (*A. flavus* ~4 log CFU/mL) inoculated on to 25 g of semi-moist pet food









MIC assay



Aspergillus flavus colonies

Mean logarithmic counts (log CFU/mL) of *A. flavus* in semi-moist pet food treatments with inclusion of liquid smokes at 0.5%, 1%, 2% and 4% concentrations in comparison with untreated (0% smoke) and positive control (potassium sorbate treatment). Limit of detection is 1 log CFU/mL for this study. Counts at 0 h indicate initial load of *A. flavus* in inoculum.









Time (days)

A. flavus counts (log CFU/mL)



Time (days)

## Conclusions

- At 4% , ~2.5 log reduction observed for Cloud S-AC15, P-1720, Cloud SC-100, Code 10 and Black deli. At 2% and 1%, Cloud S-AC15 and Cloud SC-100 had the highest reductions of ~1.7 and 1 log respectively
- Averaging across all concentrations, efficacy is:

Cloud S-AC15 > Cloud S-C100 > Code 10 > Black deli

Hickory, Code V, Purepal, and Cloud S-5 were the least effective

 Cloud S-C100 (high carbonyl) and Cloud S-AC15 (high acid, medium carbonyl) have the potential to act as an antimycotic or fungistatic at modest levels (~2%) in semi-moist pet food



Fungal growth on <u>untreated</u> sample on day 29



Fungal growth on <u>smoke-treated</u> sample on day 29

## **Experiment 2: Days to mold (shelf life) study**

### **Procedure:**

- About 30 g of semi-moist pet food sample placed in Whirl-Pak bags with 4 pin holes
- Stored at 28°C and 65-70% RH in total darkness
- Samples observed every day over 30 days for visible mold growth on the surface
- Number of days taken for the first mold colony to appear on the surface of the sample was recorded







Mean number of days taken to observe mold growth in semi-moist pet food with inclusion of liquid smoke (S1 to S8) at 0.5%, 1%, 2% and 4% in comparison to the untreated (0% smoke). Positive control (potassium sorbate treatment) did not show visible mold growth during the 30-day period (data not shown).



Each mean is based on n = 3 replications. Means among treatments across concentrations followed by different letters in lower case are significantly different (P<0.05, Tukey's test). Means among treatments within each concentration followed by different letters in upper case are significantly different (P<0.05, Tukey's test).

## Conclusions

- Liquid smoke preparations at 0.5%, 1%, 2% and 4% extended the shelf life of samples by an average of 3.9, 4.8, 9.5 and 16.4 days respectively when compared to the untreated (7.7 days)
- At 4%, smoke treatments extended shelf life of samples by up to 20 days. At 0.5% the shelf life was prolonged by only up to 5 days
- Overall, Cloud SC-100 (high carbonyl) and Code 10 (medium carbonyl, medium phenol) seemed to be the most effective in prolonging the number of days to mold by 26-28 days

## **Publications**

- Deliephan, A., Phillips, T. W., Subramanyam, B., Aldrich, C. G., Maille, J., & Manu, N. (2023). Efficacy of liquid smoke to mitigate infestations of the storage mite, *Tyrophagus putrescentiae*, in a model semi-moist pet food. Animals, 13(20), 3188.
- Deliephan, A., Dhakal, J., Subramanyam, B., & Aldrich, C. G. (2023). Effects of liquid smoke preparations on shelf life and growth of wild type mold and *Aspergillus flavus* in a model semi moist pet food. **Frontiers in Microbiology**, 14, 1154765.

## **Thank You!**



# Questions?



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