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Insights into Luncheon Meat Sample Storage and Cooking Duration with Automated Sample Preparation and Fractionated Dynamic Headspace Analysis for Maillard Reaction Products Profiling

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Keywords

Maillard Reaction Products, Luncheon Meat, Pork-Based, Chicken-Based, Plant-Based, Automated Sample Preparation, Fractionated Dynamic Headspace, Thermal Desorption, PTV Inlet, Cooled Injection System, Gas Chromatography-Mass Spectrometry

Abstract

The Maillard reaction plays a crucial role in shaping the appearance, aroma, and taste of cooked meat, with the first two factors driving initial appeal. Therefore, effectively managing the Maillard reaction is essential for gaining deeper analytical insights that support food research and development. This study aims to demonstrate the automation of handling chilled meat samples and cooking them prior to analyte extraction with dynamic headspace and chromatographic analysis. The work also highlights the capability of the GERSTEL DHS 3.5^{+} option and its unique dry purge function to extract aroma compounds from high-moisture samples, and how its sensitivity can be further enhanced using the fractionated DHS approach. Luncheon meat, chosen for its versatility, is used as the model sample in this study. The fully automated method is then applied to three variations – pork-, chicken-, and plant-based – to investigate how cooking duration affects the Maillard reaction. Sensory evaluations are also done to complement instrumental analysis and provide a comprehensive understanding of flavor development.

Introduction

Visual appearance and aroma are key attributes of meat that first captivate consumers. While some forms can be consumed raw or without further cooking, they often appear bland and lack robust flavors. Luncheon meat is a notable example of such a product. This precooked canned product, typically made from finely ground pork, starch, salt, and preservatives, was popularized during wartime for its convenience and long shelf life. Nowadays, luncheon meat can be prepared in various ways – frying, baking, braising, or boiling – which elevates its flavors and makes it a versatile ingredient in diverse cuisines. For instance, pan-fried luncheon meat becomes far more appealing with its crispy, golden-brown edges, and intensified roasted, meaty aromas. This transformation is primarily due to the Maillard reaction, a non-enzymatic reaction between reducing sugars and amino acids during cooking [1]. While other reactions, such as lipid oxidation and thiamine degradation, also contribute to flavor development [2], this work will focus on the Maillard reaction and its products.

The Maillard reaction is affected by factors such as time, temperature, pH, water activity, and the type and concentration of reactants [2], all of which impact the aroma profiles of cooked meat. The analysis of these Maillard reaction products (MRPs) can be accomplished using headspace extraction techniques like static headspace sampling (SHS), solid-phase microextraction (HS-SPME), and dynamic headspace sampling (DHS), coupled with gas chromatography-mass spectrometry (GC-MS). Among these

techniques, DHS is highly sensitive in ultra-trace analysis due to its non-equilibrium approach. It continuously purges the headspace of a sample with inert gas and traps the purged analytes on a sorbent, for example, Tenax TA. However, DHS is often less favored for high-moisture samples like cooked meat, which contains 55 - 65% water [3], as moisture build-up in the sorbent trap can interfere with the GC-MS performance [4].

The GERSTEL DHS option, detailed in AppNote 255 [5], effectively mitigates the moisture issue with its unique dry purge feature, enabling larger sample sizes without compromising the GC-MS system. The sensitivity of the DHS technique can be further enhanced using the fractionated DHS approach, which involves sequential sampling with multiple tubes under varying trapping conditions. Additionally, sample preparation, such as cooking the meats before DHS extractions, can be easily automated using the relevant modules on the GERSTEL Multipurpose Autosampler (MPS). Cooking parameters, including time and temperature, can be precisely controlled via the MAESTRO software, ensuring consistent and repeatable results.

Figure 1: GERSTEL Dynamic Headspace - DHS 3.5⁺ option.

This study introduces an innovative approach that combines the automated preparation of cooked meats with DHS-GC-MS analysis using the DHS 3.5⁺ option (Figure 1). Adapting the DHS settings from AppNote 255 [5], it investigates the impact of cooking time on the Maillard reaction in luncheon meat. The study also explores the use of fractionated DHS to enhance analytical sensitivity. Using this fully automated method, the evaluation of MRPs across different cooking durations is conducted on three types of luncheon meat – pork-, chicken-, and plant-based.

Experimental

Instrumentation

The analytical setup included the GERSTEL MPS Robotic^{Pro} Autosampler with a Peltier Cooled Stack and Agitator, the GERSTEL Dynamic Headspace (DHS 3.5⁺) option, a Thermal Desorption Unit (TD 3.5⁺), a Cooled Injection System (CIS4) equipped with Cryostatic Cooling Device (CCD2), and an Agilent 7890 Gas Chromatography (GC) system coupled with a 5977B Mass Spectrometry Detector (MSD).

Sample Preparation

Three types of luncheon meat – pork-, chicken-, and plant-based, were purchased from a local supermarket. For each sample type, 1 g was weighed into 10-mL screw neck vials and promptly stored in the Peltier Cooled Stack (Figure 2) pre-set to 4 °C to simulate the typical temperature of household refrigerators [6].

Figure 2: (left) Peltier Cooled Stack, and (right) Agitator.

As outlined in Figure 3, the following sample preparation steps were automated using the MPS to mimic the common cooking practices and ensure a realistic representation of the final dish.

1. Remove the chilled sample from the Peltier Cooled Stack and let it rest at room temperature for 6 minutes.

Chilled meat is typically left at room temperature for a specified period before cooking. This helps dissipate refrigerator odors and ensures even cooking, improving the flavor and texture of the meat. In this study, the 1 g sample took 6 minutes to reach room temperature.

2. Cook the sample in the Agitator (Figure 2) at 182 °C (360 °F).

This temperature was adapted from a recipe for luncheon meat fries. The cooking temperature and time may be adjusted based on experimental objectives.

3. Sit the cooked sample at room temperature for 5 minutes before DHS extraction.

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The resting phase after cooking helps ensure juicer, more tender, and flavorful meat. Although the optimal rest time can vary depending on the cut and cooking method, this study followed the recommended 5-minute resting period for small cuts of meat.

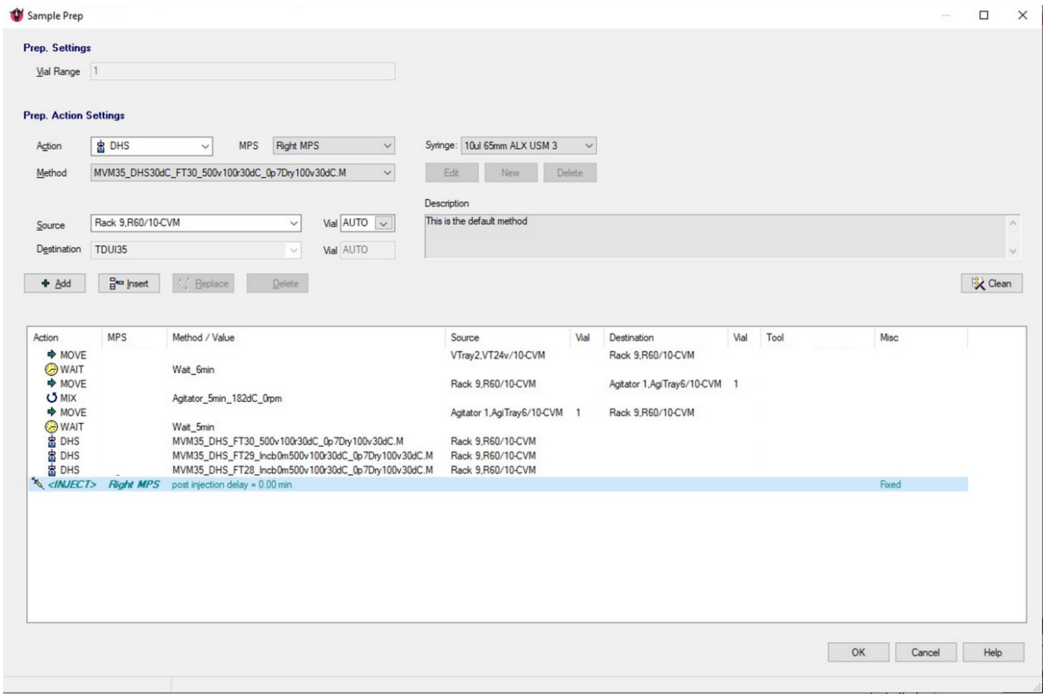

Figure 3: Automated sample preparation window. Lines 1 – 6 outline the MPS actions to cook the luncheon meat samples prior to DHS extractions.

Analysis Conditions

DHS	
Sorbent Traps	Tenax TA + TD 3.5^{+} glass tube
Incubation	60 °C (1st trap 5 min, subsequent traps 0 min)
Transfer heater	150 °C
Trap volume	500 mL
Trap flow	100 mL/min
Trap temp	30 °C
Dry volume	2972 mL
Dry flow	100 mL/min
Dry temp	30 °C

TDU	
Mode	splitless
Flow	50 mL/min
Temp	30 °C (0.01 min); 60 °C/min to 230 °C (5 min)
Transfer temp	240 °C

CIS	
Liner	Tenax TA (pre-conditioned)
Mode	solvent-venting
Split ratio	1:20
Temp	-30 °C (0.01 min), 10 °C/s to 230 °C (5 min)

Analysis Conditions GC Agilent 7890

Column	30 m ZB-WAX, d_i = 0.25 mm, d_f = 0.25 µm
Pneumatics	He; P_i = 109.38 kPa constant flow; 1.25 mL/min
Oven	50 °C (7 min), 3 °C/min to 180 °C (0 min), 10 °C/min to 230 min (5 min)

Analysis Conditions MSD Agilent 5977B

Scan	29 to 350 amu

Data Analysis

Aroma compounds in luncheon meat are tentatively identified and integrated using a combination of deconvolution (Agilent MassHunter Unknowns Analysis) and Aroma Search (Aroma Office Ver. 7) [7].

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Results and Discussion

Refrigeration – A Key Factor in Automated Sample Preparation of Luncheon Meat

The importance of refrigeration in maintaining sample integrity and enhancing analytical repeatability was demonstrated. The repeatability of aroma compounds commonly reported in cooked meats [1,8,9] was significantly improved when the pork-based samples were stored at 4 °C for approximately 12 hours before automated cooking (Table 1). The relative standard deviations (RSDs) of aroma compounds were generally below 10% across three replicates for the refrigerated samples. In contrast, most analytes from the ambient-stored samples exhibited RSDs exceeding 15%.

Table 1: Comparison of RSDs (n=3) of aroma compounds in cooked pork-based samples between chilled storage at 4 °C and ambient storage. Automated sample preparation: Cooking time, 5 min; DHS conditions: Number of Tenax TA traps, 1.

SN	Compound Name	% Relative Standard Deviations (n=3)	
		Chilled storage at 4 °C	Ambient storage
1	3-Methylbutanal	2.52	18.87
2	2,3-Butanedione	6.55	17.04
3	2,3-Pentanedione	4.92	19.72
4	Hexanal	14.24	20.25
5	Pyrazine	5.83	15.51
6	2-Pentylfuran	9.03	22.56
7	Methyl pyrazine	4.49	22.04
8	Acetoin	9.04	13.61
9	2,5-Dimethylpyrazine	12.25	24.59
10	Furfural	5.95	17.89
11	Phenylacetaldehyde	1.83	13.65
12	2-Furanmethanol	4.95	16.55
13	2,4,E,E'-Decadienal	11.40	21.71

Proper storage at 4 °C is essential not only for analytical reliability but also for food safety [6]. While canned luncheon meat is shelf-stable when sealed, it becomes susceptible to bacterial growth once opened, much like other perishable foods. Bacteria proliferate most rapidly in the temperature range between 5 °C – 60 °C, known as the 'Danger Zone' [10]. This can lead to undesirable changes in appearance, aroma, and taste. Hence, storing such samples in Peltier Cooled Stacks at 4 °C helps minimize these risks during extended automated sequence runs, maintaining sample quality and improving analytical repeatability.

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Fractionated DHS – Sequential Extractions for Enhanced Sensitivity

In this work, Tenax TA sorbent traps were used for fractionated DHS sampling to capture a wide range of aroma compounds with varying boiling points, aiming to simulate the aroma perception of a diner. Tenax TA-type sorbent is commonly used due to its high affinity for volatile and semi-volatile compounds while exhibiting low affinity for water. The same trapping and drying conditions were applied to each trap.

As shown in Figure 4, using more sorbent traps increased the extraction of aroma compounds. The single DHS extraction (i.e., one trap) from 5-minute cooked pork-based samples captured only 23 aroma compounds, while sequential extractions from the same sample with two and three Tenax TA traps detected 38 and 40 compounds respectively. Furthermore, peak responses increased with the number of sorbent traps, with three traps achieving the highest signal intensities for all aroma compounds identified. Fractionated DHS sampling with three traps also demonstrated satisfactory repeatability across three replicates, with most of the 40 compounds exhibiting RSDs below 15% and an average RSD of 14.6%.

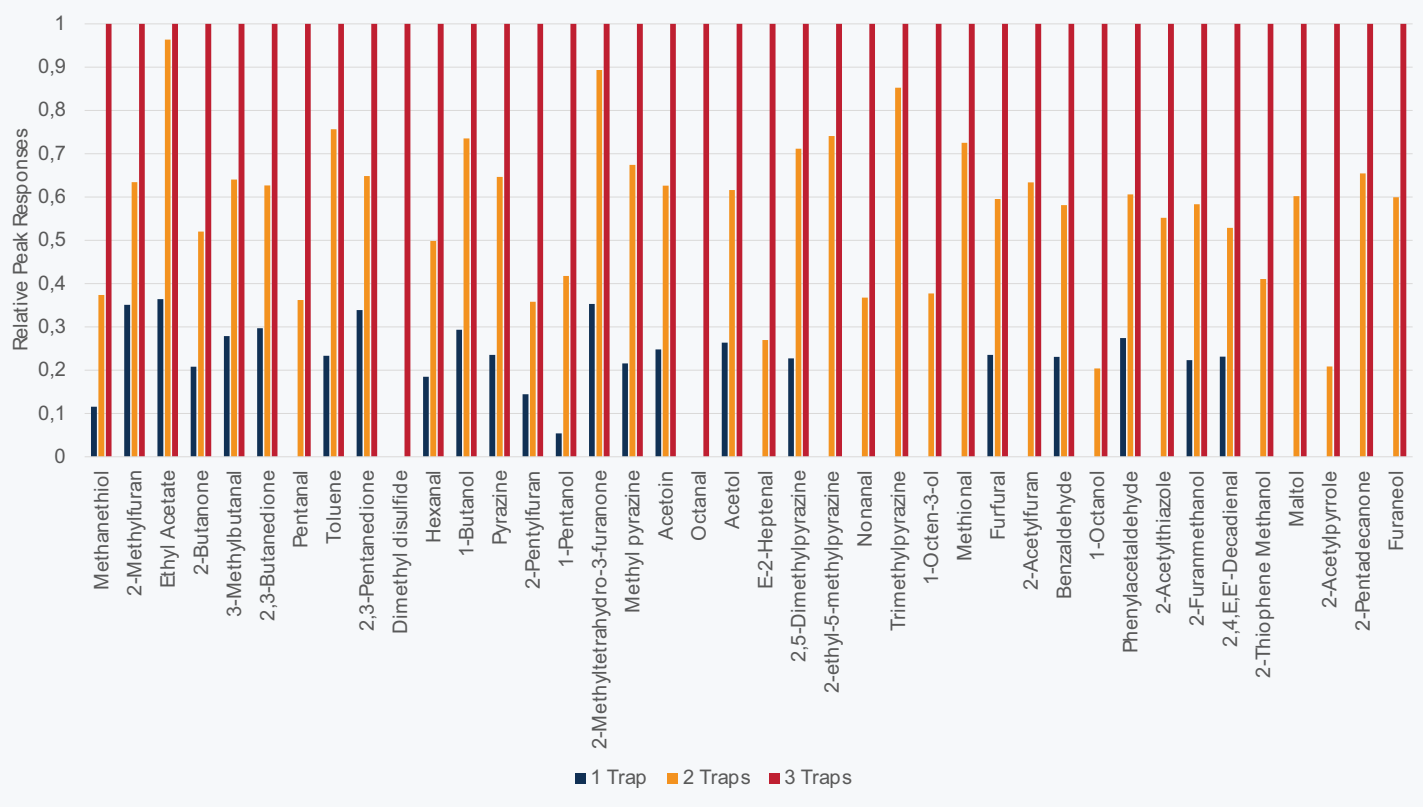

Figure 4: Comparison of relative peak responses of aroma compounds (n=3) identified in cooked pork-based samples across different number of Tenax TA traps for fractionated DHS sampling. Automated sample preparation: Cooking time, 5 min.

While the fractionated DHS approach enhances sensitivity, users should still assess its applicability case-by-case. For instance, sequential sampling may alter aroma profiles due to prolonged sample incubation, which might not align with the intended analytical objectives. Additionally, despite using the <PrepAhead> function, the analysis throughput may reduce depending on the duration of the GC-MS run.

Effects of Cooking Time on the Maillard Reaction

Automated cooking prior to fractionated DHS sampling with three Tenax TA traps was employed to investigate the effects of cooking time on the Maillard reaction in pork-, chicken-, and plant-based luncheon meat. Samples were analyzed at different cooking stages: uncooked, cooked for 5 minutes, 10 minutes, and 15 minutes. Notably, the uncooked samples were incubated at 30 °C instead of 60 °C before extractions to reflect the ambient temperature in Southeast Asia.

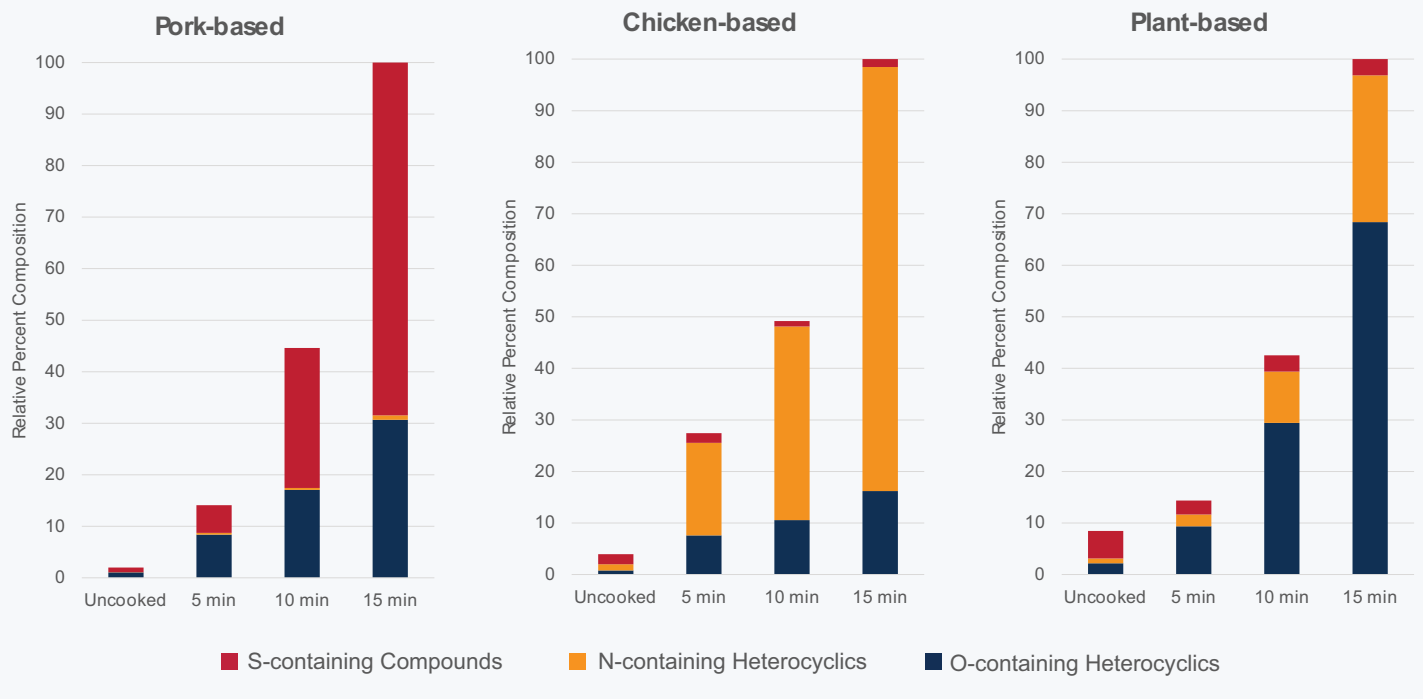

Figure 5: Relative percent composition of MRPs in each type of luncheon meat across different cooking durations (n=3). DHS conditions: Number of Tenax TA traps, 3.

The Maillard reaction mainly produces three classes of compounds – sulfur-, nitrogen-, and oxygen-containing – which contribute significantly to cooked meat aromas [11]. As the cooking duration extended, the amount of MRPs increased in all three types of luncheon meat (Figure 5) and potentially led to more intense aromas. In addition, the main compound class of MRPs differed across the three types of luncheon meat. S-containing compounds, such as thiophenes, thiazoles, and thiols, were most abundant in pork-based samples and they usually impart sulfurous, onion-like, meaty aromas. Chicken-based samples predominantly contained N-containing heterocyclics, such as pyrazines, associated with nutty and roasted aromas. Plant-based samples were characterized by O-containing heterocyclics like furans, furanones, and pyrans, which contributed caramel-like aromas. These compositional differences likely influenced the resulting aroma profiles of each sample type, which could impact consumer perception. The corresponding chromatograms for the 15-minute cooked samples are shown in Figure 6, and the MRPs are highlighted in brown.

Generally, the analytical workflow of food product development requires both instrumental analysis and sensory evaluation. Other than enhancing the formation of MRPs, increasing the cooking time also affected the visual characteristics of the samples. The Maillard reaction, also known as the 'browning' reaction, produces melanoidins – brown pigments responsible for the color of cooked meats. Prolonged cooking can intensify this browning, resulting in an excessively dark or 'burnt' appearance which can negatively impact its visual appeal and perceived edibility. Therefore, the color changes in the luncheon meat samples across different cooking times are shown in Table 2, along with aroma assessments from two panelists.

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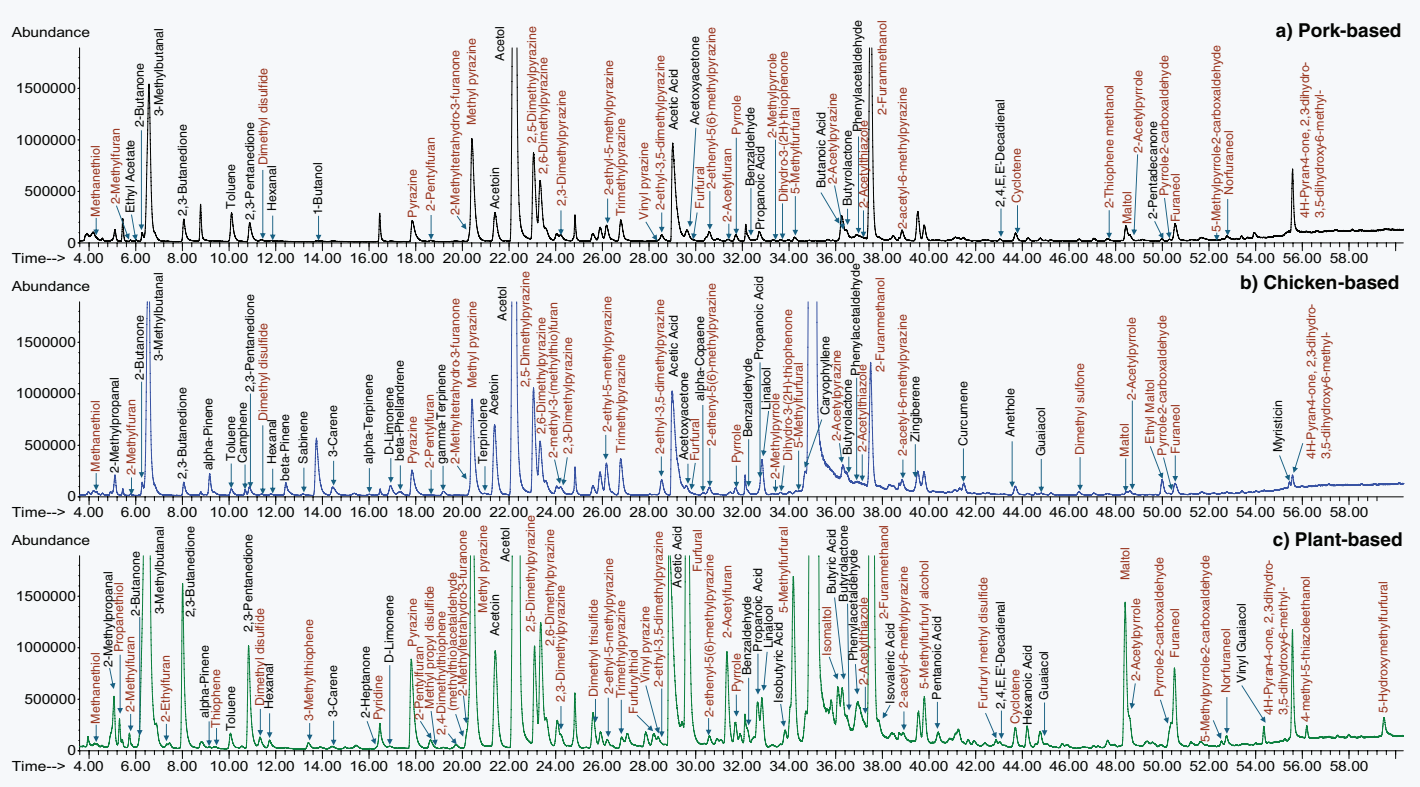

Figure 6: Stacked view of TICs for each type of 15-minute cooked luncheon meat sample. a) Pork-based, b) Chicken-based, and c) Plant-based. The TICs only displayed labeled analyte peaks reported in other studies on cooked meats [1,8,9,13]. MRPs are highlighted in brown font.

Table 2: Sensory evaluations (based on senses of sight and smell) of different types of luncheon meat across different cooking durations. The light blue-shaded boxes indicate the preferences of each panelist for the cooking time of each sample.

Cooking Duration at 182°C	Uncooked (Original State)		5 minutes		10 minutes		15 minutes	
	Appearance	Aroma	Appearance	Aroma	Appearance	Aroma	Appearance	Aroma
Pork-based		**Panelist A:** Mild; Greasy; Not fresh **Panelist B:** Mild; Sausage/ham		**Panelist A:** Meaty; Greasy **Panelist B:** Sausage; Processed meat		**Panelist A:** Meaty; Fragrant; Less greasy **Panelist B:** Burnt processed meat; Salty		**Panelist A:** Nutty; Burnt; Garlic-like **Panelist B:** Bitter; Burnt; Roasted; Meaty
Chicken-based		**Panelist A:** Chicken ham; Mild; Salty **Panelist B:** Salty; Sausage		**Panelist A:** Chicken ham; Salty **Panelist B:** Very salty; Chicken sausage		**Panelist A:** Chicken ham; Less salty **Panelist B:** Very salty; Chicken sausage; More aromatic		**Panelist A:** Chicken ham; Salty **Panelist B:** Not as salty; More aromatic; Chicken sausage
Plant-based		**Panelist A:** Soy; Beany **Panelist B:** Salty; Green pea		**Panelist A:** Beany; Boiled vegetable **Panelist B:** Beany; MSG/seasoning		**Panelist A:** Beany; Fragrant **Panelist B:** Very burnt; Salty; Smelly; Beany		**Panelist A:** Beany; Burnt; Roasted **Panelist B:** Stronger beany; Seasoning; Burnt

From a consumer perspective, both panelists agreed that the uncooked versions of all three types were the least appealing due to their pale appearance and mild aroma. As the cooking time extended, both panelists noted more intense aromas, with 'meaty', 'roasted', and 'burnt' notes becoming more pronounced. This aligned with the higher levels of MRPs produced at longer cooking durations. Both panelists also noted that each type of cooked sample exhibited distinct aromas, which again corresponded with instrumental analytical findings showing different MRP profiles. Although the longest-cooked samples had the most intense aroma, they were not necessarily the most appealing. The light blue-shaded boxes in Table 2 indicate the differing opinions of the panelists on the cooked samples. Panelist B, in particular, found none of the cooked plant-based samples appetizing. These sensory evaluations highlight how cooking time influences consumer preferences, emphasizing the need to optimize both visual appeal and the formation of flavor-enhancing compounds in food product development with instrumental analytical and sensory findings working in tandem [12].

Conclusions

This work demonstrates an innovative, fully automated method for preparing cooked meats, followed by sequential DHS extractions using the GERSTEL DHS 3.5^{+} option with its unique dry purge function to analyze aroma compounds in these high-moisture samples. Automation, including having precise control over temperature and time, improves analytical repeatability and enables effective management of critical flavor development pathways, such as the Maillard reaction. Additionally, the fractionated DHS approach has been demonstrated to enhance the sensitivity of the DHS technique, further improving its overall effectiveness. Consequently, the insights gained from this instrumental analytical approach on the three variations of luncheon meat cooked for different durations complement sensory evaluations and potentially provide valuable support to food research and development.

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