SYMCEL

ADVANCEMENTS IN FOOD SAFETY: ISOTHERMAL MICROCALORIMETRY FOR DETECTING BACTERIAL SPOILAGE IN MINCED CHICKEN

INTRODUCTION

The detection of bacterial levels in complex food products such as minced meat is a critical aspect of food production, essential for preventing spoilage and ensuring food safety. Contamination in products can lead to significant public health risks, including foodborne illnesses caused by pathogens such as *Campylobacter* ssp. and *Salmonella* ssp. Additionally, bacterial spoilage not only affects the quality and shelf life of meat products but also contributes to unsustainable food waste and its associated economic and environmental implications[1].

Traditional methods for detecting bacterial contamination in food products often involve lengthy and complex processes, including sample preparation, culturing, and microbial enumeration. These methods, while reliable, are time-consuming and may delay critical decisions in the food production process. In a fast-paced production environment, the need for rapid and accurate detection methods is paramount to prevent further spoilage, enhance food safety, and minimize waste.

Isothermal microcalorimetry presents a promising solution to thesechallenges by providing a fast, efficient, and direct method for detecting bacterial load on minced meat. This innovative technology measures the metabolic heat flow of bacterial activity, allowing for real-time assessment of bioload and contaminants directly on the product material based on the time to detection of activity. Unlike conventional techniques such as CFU plating and Q-PCR, microcalorimetry requires no sample preparation, significantly reducing the time and effort needed for detection. Non-sterile products like minced meat are expected to contain a norme-biotic load of microbes. Depending on their quantity, a faster or slower detection of activity can be expected with a microcalorimeter. In most cases, the main microbiome will consist of aerobic mesophilic bacteria, like *E.coli*, as well as anaerobic bacteria, such as lactobacillus and *Lactococcus* ssp.

The metabolic signal of the combined community can be detected as a whole for any product. The metabolic output of each community or species will give a unique but reproducible metabolic pattern over time in a micro-calorimeter. This also means that contamination with foodborne pathogens could potentially be seen as a change of the unique metabolic pattern of the norme-biotic microbes in the product. Here, we used minced chicken meat as a model for detecting microbial activity in a non-sterile food product.

PROCEDURE

Fresh minced chicken meat bought in a local store one day from production was transferred in portions of 400 µg directly into the titanium calVials, whereas a portion of the same batch had been left at room temperature (~20 °C) for 12 hours before being transferred into the vials. The metabolic output from the samples was registered over 20 hours at 37 °C.

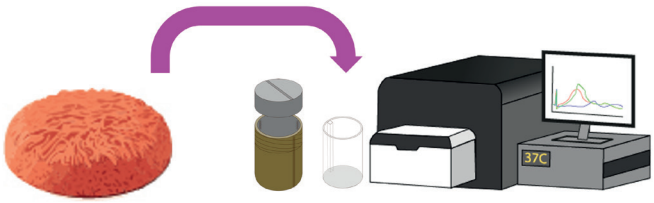

Figure 1 Schematic drawing of the process. Raw minced chicken meat added directly into the calScreener titanium vials placed into the instrument for evaluation.

In an effort to detect contamination by a food pathogen, *Salmonella enterica* was spiked into samples of fresh minced chicken meat at a level of 15 CFU per sample, after which the samples were measured in the calScreener at 37 °C for 20 hours.

RESULTS

It was possible to detect a sustainable growing meta-bolic signal approximately 5.5 hours after the start of incubation in fresh minced meat, whereas the signal could be detected immediately in minced meat left at room temperature for 12 hours to spoil. The disparity between the time to detection of activity can be directly correlated to the starting microbial load, indicating a very low bacterial load in the fresh meat compared to the spoiled sample (Fig. 2).

Direct detection of microbial activity in minced chicken meat

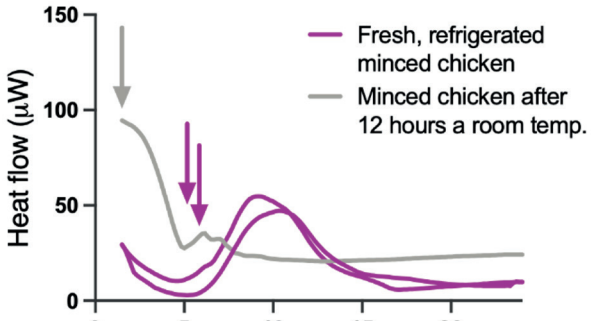

Figure 2 Metabolic thermogram from pure minced chicken meat. In fresh refrigerated meat a signal could the identified after 5.2 and 5.5 hours respectively (indicated by purple arrows). In spoiled meat the detection was instant (indicated by gray arrows).

With the introduction of *S.enterica* directly into the meat in 10-fold dilutions, the time to detection of a sustainable signal decreased almost linearly with increasing amounts of CFU. As the fresh meat

was spiked with a very low amount of *S.enterica* (15 CFU and ~1.5), the time to detection did not change compared to fresh meat on its own, indicating that the original amount of microbiomes in the fresh sample might be in the tens of CFU. But with the introduction of a small amount of *S.enterica*, the metabolic signature of the samples changed significantly, clearly indicating the introduction of the contaminant (Fig. 3).

Detection of salmonella contamination with a linear correlation to number of bacterial cells

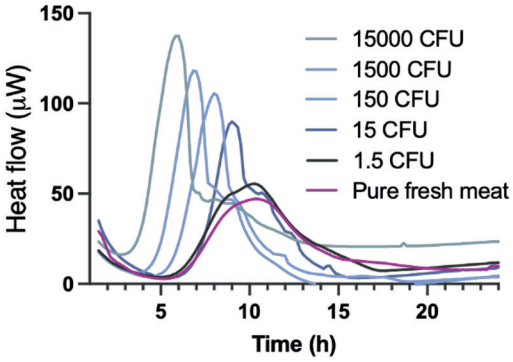

Figure 3 Metabolic signal from pure fresh refrigerated meat or fresh meat spiked with 10-fold dilutions of S.enterica. The time to detection of activity decreases linearly with increasing quantities of spiked cells. At the lowest cell inoculum, the signal has the same time to detection as the unspiked meat.

CONCLUSION

The application of isothermal microcalorimetry to detect food spoilage in minced chicken meat represents a significant advancement in microbial detection methods.

This study demonstrates that isothermal microcalorimetry can effectively measure metabolic activity, allowing for the rapid assessment of bacterial contamination. The technology successfully distinguished between fresh and spoiled meat sam-ples based on the time taken to detect metabolic activity, correlating with microbial load.

Additionally, the introduction of *S. enterica* showed that even low levels of contamination could be detected, indicating the method's sensitivity. These findings suggest that the calScreener is a promising tool for improving food safety and reducing food waste through timely and accurate microbial detection.

References

[1] Manpreet Singh et al., Emerging Meat Processing Technologies for Microbiological Safety of Meat and Meat Products. Meat and Muscle Biology. 2020

[2] Vasavada, P.C., Conventional and Novel Rapid Methods for Detection and Enumeration of Microorganisms, Food Safety Engineering, 2020.

[3] Michael Tosin Bayode et al. Isothermal microcalorimetry (IMC) calScreener: automated peculiarities of antimicrobial therapy and metabolism depth of multidrug resistant bacteria, Bulletin of the National Research Centre, 2022