Application of Isothermal Microcalorimetry to MST Products – A New Method to Monitor Aging

Robert B. Ragland and Mark J. Rusyniak
Altria Client Services LLC, Richmond, VA 23219

73rd TSRC - Tobacco Science Research Conference
September 15-18, 2019, Leesburg, VA, USA

WWW.ALTRIA.COM/ALCS-SCIENCE

Altria

ABSTRACT

Isothermal microcalorimetry can be used to measure heat conduction as a function of time as a tool to rapidly measure chemical and physical changes that can occur during aging. This can be particularly useful when trying to determine a product's stability over its shelf life which typically involves the collection of a large amount of data at designated time intervals to assess changes in the finished product, as a function of time and environmental conditions. For tobacco products such as moist smokeless tobacco (MST), it can be difficult to attribute chemical or physical changes to a singular mechanism due to chemical reactions and microbial activity occurring concurrently.

Commercially available MST products were tested to determine characteristic heat flows relative to tobacco particle size and flavor variants. Also, samples of the unfinished MST tobacco base and finished product were treated using electron beam irradiation for the purpose of cold pasteurizing the material. The pasteurized samples were analyzed against non-pasteurized variants to assess heat flow contribution due to microbial activity and lignin oxidation. Results showed that products pasteurized prior to fermentation had a reduced heat flow signal similar to products pasteurized after fermentation indicating the microbes used for fermentation do not negatively impact product stability. Samples were also tested in ampoules pressurized with nitrogen and oxygen to measure heat flow relative to oxygen consumption and transitions to anoxic conditions. Based on pressure calorimetry, oxygen consumption appears to constitute a large portion of the baseline heat flow measurements where relatively small heat flows were observed in an inert atmosphere.

INTRODUCTION

Determination of the kinetics and stability of tobacco products at room temperature is critical but usually involves a large time and financial commitment. Common artificial aging methods (e.g. accelerated) utilize elevated temperatures to obtain quantifiable data quicker; however, it is only valid if the reaction mechanism is independent of the temperature range.

Isothermal microcalorimetry can measure the rate of heat released due to chemical or physical processes in real time with sensitivity down to the µW-nW range. The sensitivity of the instrument allows for kinetic measurements at the start of the reaction where heat production is typically greatest without having to wait weeks or months to get insightful data.

OBJECTIVE

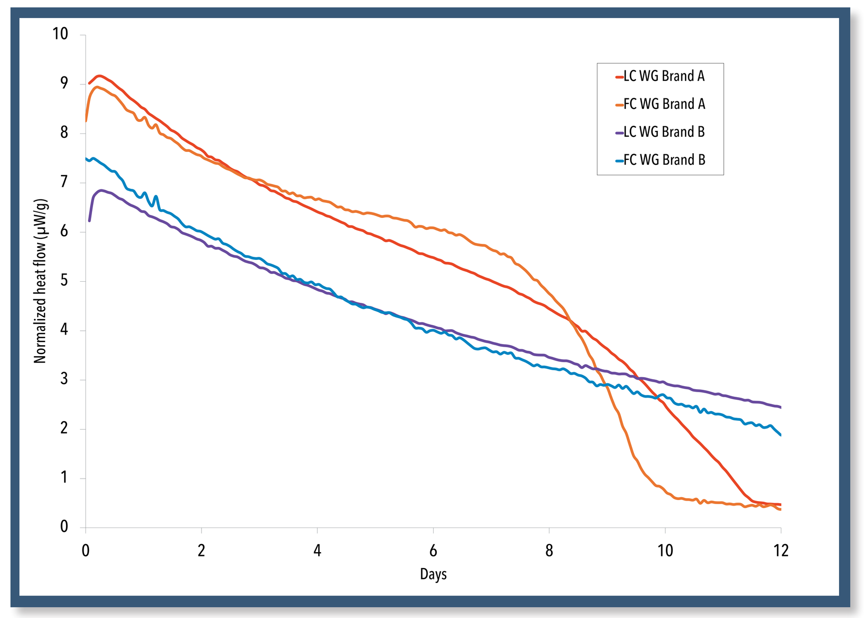
Develop a method to rapidly screen tobacco products and materials

- Observe kinetics and heat flow of different flavor groups
- Observe kinetics and heat flow of different cut sizes relative to surface area to volume
- Determine if heat flux is attributed to oxygen concentration
- Isolate heat flux contribution from microbial activity and oxidation

METHODS

- TA Instruments multichannel Thermal Activity Monitor (TAM) equipped with a vacuum/pressure multicalorimeter was used to measure heat flow as a function of time and atmosphere conditions at 25°C
- Tests were performed using three commonly produced flavors and two different cut sizes of commercially available MST
- Samples were balanced to within ± 20% of the internal fixed reference heat capacity to reduce baseline noise
- Characteristic heat flow data was collected by hermetically sealing MST in glass ampoules
- Modified atmosphere data was collected using 20mL stainless steel vacuum/pressure ampoules and pressurizing with gas to 5 bar
- Reaction rate order constants were obtained using measured heat (J) and heat flow (W) signals
- With the assumption that all of the enthalpy is attributed to oxidation, the oxycaloric equivalent of Thornton's constant (-455 ± 15kJ mol^{-1}) can be used to determine the concentration of oxygen reacted
- Heat flow signals (µW) were normalized to a per gram basis for effective analysis and comparative purposes

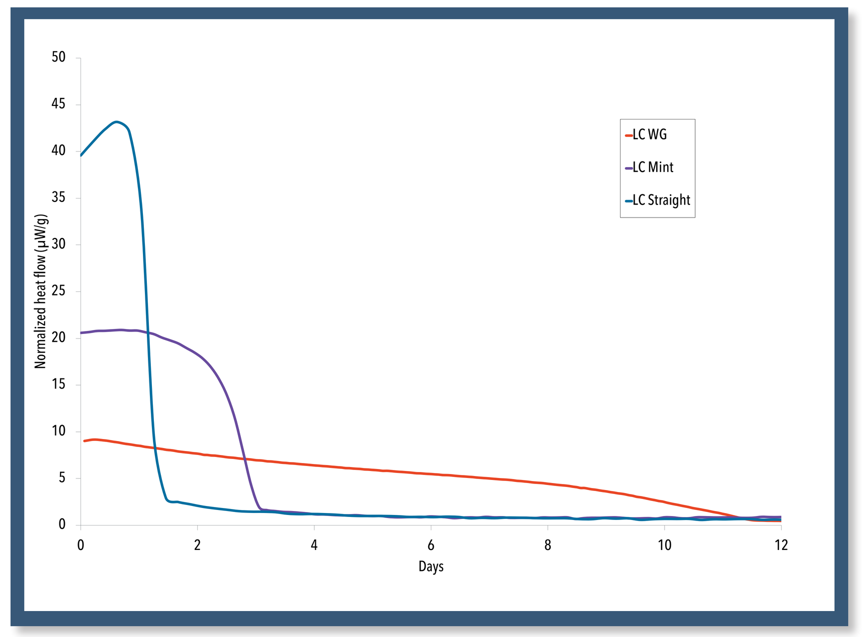
RESULTS



Brand A and Brand B Commercial Long Cut (LC) and Fine Cut (FC) Wintergreen MST Normalized Heat Flow

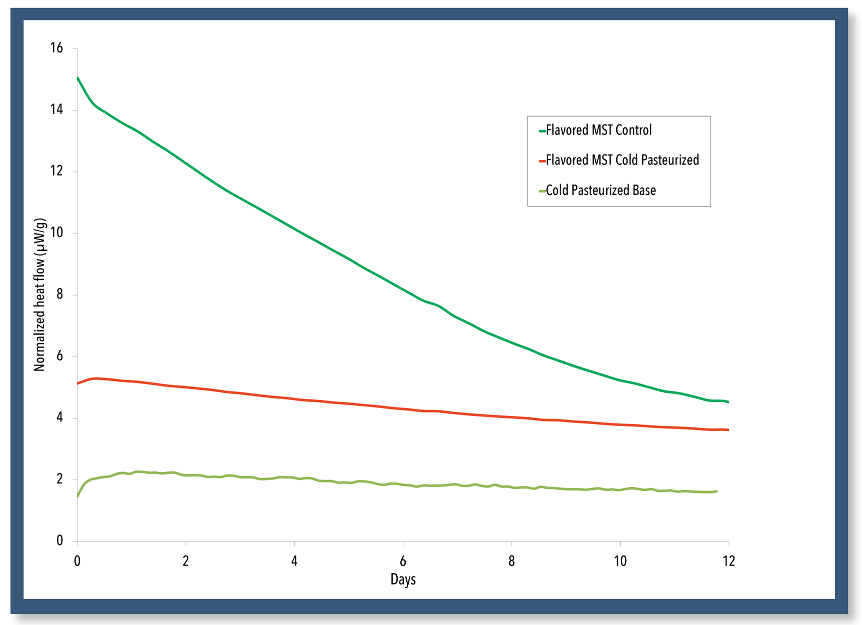
- Two different brands of MST show two distinct characteristic heat flows
- Visible shoulder in heat flow signal where oxygen is depleted in Brand A but not Brand B
- Particle size does not appear to affect the kinetics of aging

Product	Particle Size (µm)	Mass MST (g)	Integrated Heat (J)	Normalized Heat (J/g)	Moles O_2 Reacted	Moles O_2/g MST	k (s^{-1})
Long Cut Wintergreen A	1200-1400	10.103	53.676	5.313	1.18E-04	1.17E-05	6.415E-07
Fine Cut Wintergreen A	600-750	1.982	10.460	5.277	2.30E-05	1.16E-05	7.335E-07
Long Cut Wintergreen B	1300-1400	9.742	43.644	4.480	9.59E-05	9.85E-06	9.937E-07
Fine Cut Wintergreen B	500-600	2.049	9.242	4.511	2.03E-05	9.92E-06	1.180E-06



Commercial Long Cut Wintergreen, Mint, and Straight Flavored MST Normalized Heat Flow

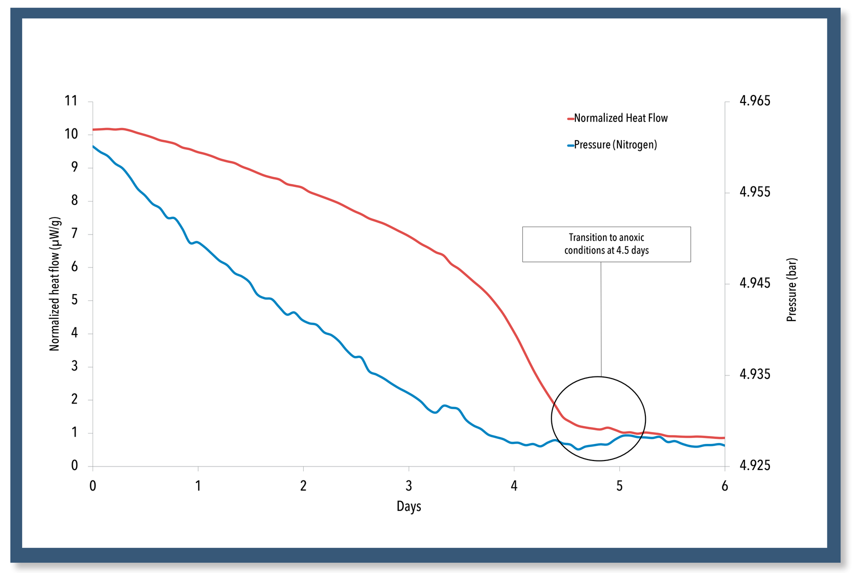
- Three different flavors of Long Cut MST from one brand show three distinct characteristic heat flows
- Visible shoulder in heat flow signals where oxygen is depleted at 3 different time points
- Heat flow signals indicate different flavor systems may consume oxygen at different rates



Normalized Heat Flow of Commercial Long Cut Flavored MST and a Cold Pasteurized Variant

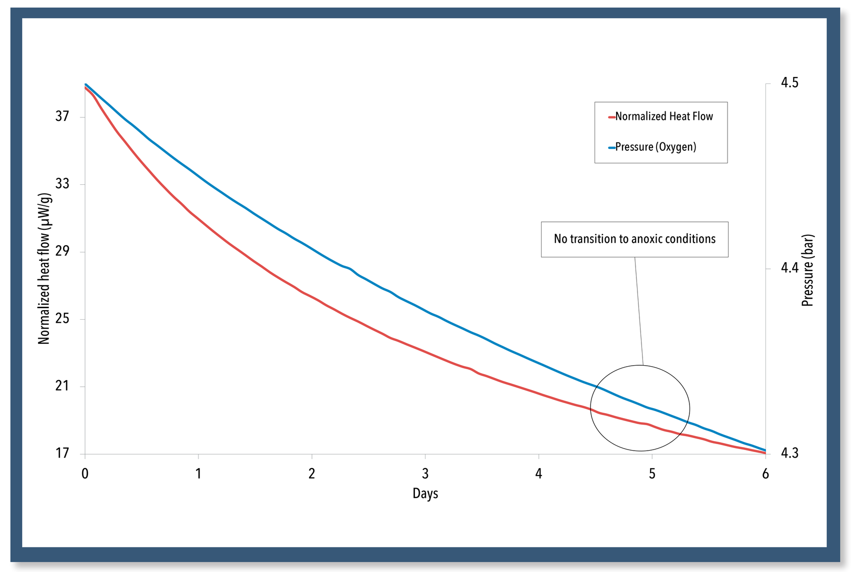
- The cold pasteurized base material (cut tobacco) shows a heat flow of 2µW/g at a relative steady state
- The control shows a higher initial heat flow signal that decreases as oxygen is consumed
- The cold pasteurized variant (same flavor as control) shows a slightly higher heat flow than the base material which can be attributed to the flavor application

RESULTS



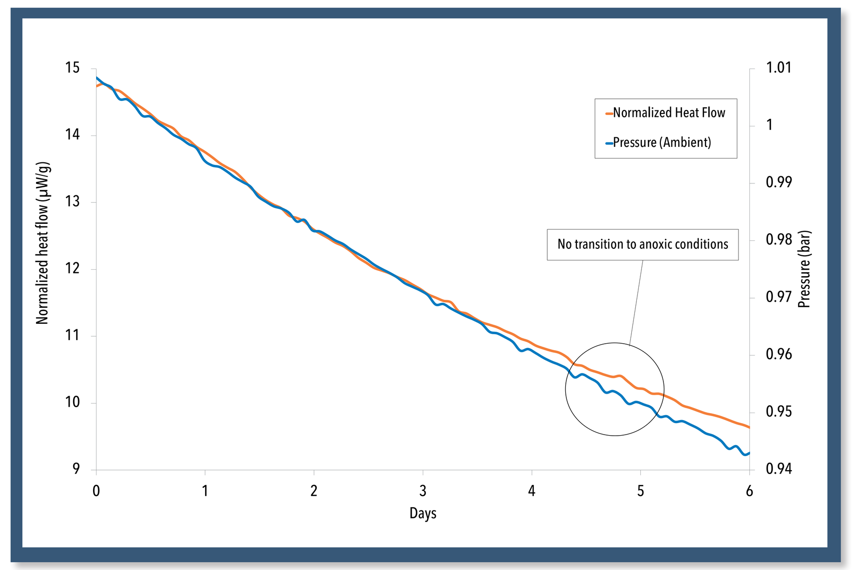
Commercial MST in Stainless Steel Ampoule under 5 bar Pressure (Nitrogen)

- ΔP – 0.03 bar
- Integrated Heat – 19.162 Joules
- Visible shoulder in heat flow signal where oxygen is depleted
- Heat flow signal and pressure signal approach steady state conditions



Commercial MST in Stainless Steel Ampoule under 5 bar Pressure (Oxygen)

- ΔP – 0.20 bar
- Integrated Heat – 80.025 Joules
- Oxygen is abundant so there is no stabilization of the heat flow or pressure signal to steady state
- Relatively large drop in pressure and high heat flow signal indicate oxygen is being consumed



Commercial MST in Stainless Steel Ampoule under Ambient Pressure

- ΔP – 0.07 bar
- Integrated Heat – 35.634 Joules
- Oxygen is still available in the headspace so there is no transition to anoxic conditions in the same time period
- Pressure drop is higher than N_2 but lower than O_2

Product	Mass MST (g)	Integrated Heat (J)	Moles O_2 Reacted	k (s^{-1})
Long Cut Wintergreen 5 bar N_2	5.4539	19.162	4.21E-05	1.49E-06
Long Cut Wintergreen 5 bar O_2	5.8814	80.025	1.76E-04	1.92E-06
Long Cut Wintergreen Control	5.2827	35.634	7.83E-05	8.32E-07

CONCLUSIONS

- The results suggest that heat flow due to oxygen consumption can be measured and kinetic information can be derived
- Particle size does not appear to notably affect the reaction kinetics
- Reaction kinetics appear to vary depending on the applied flavor. Further work is needed to determine the impact of flavor on the aging kinetics
- Microbes used for fermentation do not appear to notably impact the measured heat flow

STRENGTHS & LIMITATIONS

- This technique allows for real-time kinetic behavior to be obtained, facilitating an enhanced understanding of the product and rapid screening.
- Isothermal Microcalorimetry is not a specific technique so it only gives the net kinetic behavior. Since it is non-destructive, this technique allows for further analytical exploration (e.g. chromatography or spectroscopy).

REFERENCES AND NOTES

- Beezer AE, Gaisford S. 2013. Isothermal Microcalorimetry in the Pharmaceutical Sciences. European Pharm Rev, 18(3) 64-8.
- Hansen LD, Lewis EA, Eatough DJ, Bergstom RG, DeGraft-Johnson D. 1989. Kinetics of Drug Decomposition by Heat Conduction Calorimetry. Pharmaceutical Res, 6(1).
- Hansen LD, Macfarlane C, McKinnon N, Smith BN, Criddle RS. 2004. Use of Calorespirometric Ratios, Heat Per CO_2 and Heat Per O_2, to Quantify Metabolic Paths and Energetics of Growing Cells. Thermochimica Acta, 422, 55-61.
- Almqvist SO, Dunas LK, Rorstrom AO, Wiernik A. 1991. Microcalorimetry: A Useful Tool for Solving Shelf Life Problems in the Food Industry. Thermometric, Application Note 22016.