

2006 MAFMA Final Report

Project Title: **Mechanism of Action of Menthone in Ameliorating Oral Cavity Malodor caused by Consumption of Garlic**

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Please complete all questions below and attached form

1. Objective Summary

The main goal of this project was to determine the possible mechanism(s) of action of menthone in controlling selected volatile sulfur compounds (VSCs) responsible for garlic breath. To identify the mechanism by which menthone ameliorates garlic breath, several hypotheses on the possible modes of action were considered:

- 1) Menthone may affect odorant partitioning, thus reducing the amount of VSCs which volatilize in the oral cavity.
- 2) A secondary equilibrium may exist between menthone and VSCs. In this case, sulfur compounds may solubilize in menthone, which is hydrophobic like most of the VSCs, and by solubilizing the VSCs menthone reduces their headspace concentrations.
- 3) A reaction may occur directly between menthone and the VSCs, thus reducing the concentration of all reactants, in particular the VSCs.

2. Objective Accomplishments

After analyzing all the hypotheses, we have compelling evidence to show that the mechanism of action of menthone in reducing VSC from garlic is not a direct reaction, since no additional semi-volatile or nonvolatile compounds were formed by reaction (hypothesis 3). Instead, menthone appears to affect the partitioning of VSCs between the liquid and the gas phases, thereby reducing the amount of VSCs present in the headspace (hypothesis 1). After menthone was added to a DAD solution, ~94% of DAD was found in the menthone layer, and only ~1% in the water layer. This suggests that the mechanism of action of menthone in reducing VSCs is by affinity between menthone and diallyl disulfide (DAD) that can create a secondary equilibrium, thus reducing the DAD available to volatilize into the headspace (hypothesis 2).

Approach to test the hypotheses:

Hypothesis 1:

To assess this hypothesis we first developed and standardized procedures for static headspace – GC and then determined partition coefficients (K) for diallyl disulfide (DAD) and allyl methyl sulfide (AMS) with and without menthone in a simple matrix (water) following the Phase Ratio Variation Method (Ettre et al., 1993). Static headspace-gas chromatography was conducted using Gerstel MPS2 equipped with static headspace capability and an Agilent 6890 GC with flame photometric (FPD, sulfur detector) and flame ionization (FID) detectors. Solutions of 10 ppm of

VSCs were prepared. Different volumes (1,2,3,4 mL.) of this solution were analyzed at the same temperature (37°C). Linear regression analysis was performed with the data and partition coefficient was calculated from the linear regression equation: $y = a + bx$, where $x = \beta$, $y = 1/\text{peak area}$ and $K = a/b$. The partition coefficient (K) was calculated for diallyl disulfide (DAD) and allyl methyl sulfide (AMS) with and without menthone to compare the K values and determine if there was some change. Results showed an overall reduction of VSCs after menthone addition. DAD showed greater reduction in headspace than AMS after menthone addition.

Hypothesis 2:

To address hypothesis 2, a mass balance of DAD was performed. Ten ppm of DAD solution was prepared in deodorized water. Then 10 mL of the solution were mixed with 100uL menthone, incubated at 37°C for 20 minutes in a water bath and then shaken for 10 minutes at 200rpm on an orbital shaker (VWR Scientific Product, West Chester, PA). Then the solution was centrifuged for 10 minutes at 2000 rpm on a Damon IEC HN-SII centrifuge and menthone layer was separated from water layer. Each phase was extracted with 1mL diethyl ether and then concentrated to 100 uL. A control sample containing only DAD in water was extracted in the same way as the other samples.

Samples then were analyzed by gas-chromatography – mass spectrometry (GC-MS) using a 6890 GC/5973 mass selective detector with a flame-ionizing detector and cold splittless injection mode. 1 uL of each sample was injected and the experiment was run in duplicate. Results showed that after menthone addition, most of DAD ~94% (originally in the water phase) was then found in the menthone. Approximately 1% of DAD was found in the water phase. The 5% DAD not recovered from any of the phases may be due to experimental error or the high volatility of sulfur compounds.

Hypothesis 3:

To test if a direct reaction occurred between menthone and the VSCs, we looked for the formation of additional semi-volatile and volatile compounds. Solutions of 10 ppm DAD and AMS were prepared and analyzed with and without menthone. To look for volatiles formation, static headspace GC-MS and solid phase microextraction GC-MS (85 uL Carboxen/PDMS StableFlex fiber) were used. Results suggested a 100% reduction of DAD headspace after menthone addition, and ~78% AMS reduction. However, no additional volatile compounds were detected. To determine if any semi-volatile compounds were formed, 10 mL of a 10 ppm DAD solution, with and without menthone, were extracted with diethyl ether (three times) and dodecane used as internal standard. Then 1 uL from the extraction was injected on column in the GC-MS 6980 GC/5973 mass selective detector with a flame-ionizing detector. Results showed no additional semi-volatile compounds were formed after menthone was added to the DAD solution. To verify that no reaction was occurring and that nonvolatile compounds were not being formed, nuclear magnetic resonance (NMR) and infrared spectrometry (IR) analysis were conducted. ^1H NMR spectra were recorded on a Varian Inova (500 MHz, ^1H) and a Varian Unity-500 (500 MHz, ^1H , 126 MHz, ^{13}C) spectrometer in chloroform- d using chloroform (7.27 ppm, ^1H , 77.23 ppm, ^{13}C) as an internal reference for ^1H . Infrared (IR) spectra were as thin films (neat) in NaCl cells, using a Perkin Elmer Spectrum BX spectrophotometer. Results confirmed that there is no reaction occurs between DAD and menthone.

3. Unexpected findings, if any

Initially, the hypothesis of a direct reaction between menthone and VSCs seemed to be the most likely to happen. However, we were surprised that no nonvolatile adducts were formed even with the decrease in DAD after menthone addition.

4. Practical impacts of research efforts. Include: implementation of accomplishments by industry partners (if any), identification of economic impacts, and any further pursuit by PI of research in area of this project whether MAFMA or not.

a. Short Term Impacts – Wrigley is currently using Menthone in some of their breath control products (in particular to reduce sulfurous odors)

b. Long Term Impacts – Knowledge of the mechanism of action of Menthone in reducing garlic odorants will lead to discovery and development of additional breath control agents based on monoterpenes.

5. If you are also making reports to other funding agencies in the course of this research work, please include a copy of that report.

A second update (oral report) for the project was presented to Wm. Wrigley, Jr. Company on May, 2007.

6. If any publications resulted from the research, a copy must be included.

Publications:

Thesis: Carrasco-Hernandez, G.M. *Efficacy of various natural essential oil compounds in the control of oral cavity malodor caused by consumption of garlic and possible mechanism of action.* MS Thesis, University of Illinois, December 2007.

Presentations:

Carrasco, G. and Cadwallader, K.R. 2007. Mechanism of action of menthone in ameliorating oral cavity malodor caused by consumption of garlic. 234th ACS National Meeting, Boston, MA, August 19-23. (poster presentation)

Carrasco, G. and Cadwallader, K.R. Mechanism of action of menthone in ameliorating oral cavity malodor caused by consumption of garlic. Presented in the graduate student paper competition (oral paper) at the Society of Hispanic Professional Engineers (SPHE) National Conference, Philadelphia, PA, October 31- November 3, 2007. [Gabriela Carrasco won first place.]

Carrasco, G. and Cadwallader, K.R.. Headspace reduction of the main volatile sulfur compounds responsible for garlic breath by different terpenoids. Accepted for presentation at the Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 28 – July 1, 2008.