Measurement of Minimum Inhibitory Concentration (MIC) of Individual and Combinations of Essential Oil Volatiles in Food

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Abstract

The use of essential oil volatiles as natural food preservatives has received significant attention in recent years. Shelf life extension can be achieved through the appropriate design of active packaging systems that release volatiles into the product headspace at controlled rates. In some applications, the volatile concentrations required to delay or prevent spoilage can cause sensory changes to the product. The use of multiple volatiles and the potential for synergistic effects offer opportunities to minimise sensory effects in the pursuit of shelf life extension. The design of these systems requires a good knowledge of the target headspace volatile concentrations required to inhibit growth.

The aim of this research was to analyse the methods for measurement of minimum inhibitory concentration (MIC) for individual and mixtures of essential oil volatiles in food systems. Carvacrol and thymol, the predominant phenolic constituents of *Origanum vulgare* and *Thymus vulgaris* were selected as example active agents. Both are known to have strong antimicrobial activity.

The accuracy of the techniques normally applied to measure MICs against targeted microorganisms in the headspace is questionable. Volatile compounds released into the test headspace are absorbed by media and dish materials and lost into the environment. As such the MIC data collected are difficult to interpret and can even exceed saturated vapour pressures of the volatile compounds.

To demonstrate this, the Petri dish reversed method headspace dynamics was characterised during standard MIC tests. Factors influencing the sorption of volatiles by the culture media (Potato Dextrose Agar) and other parts of the system were investigated. The concentrations of antimicrobial compounds in the headspace were quantified using gas chromatography-mass spectrometry (GC-MS). The study showed that very low concentrations were found during MIC measurement and that the concentrations changed dynamically during the incubation period. The results demonstrated that absorption of vapour by the Potato Dextrose Agar (PDA) strongly influenced the headspace dynamics and was the main reason for the low volatile concentrations in the headspace.

The partitioning of carvacrol and thymol ($K_A/_W$ values 5.94×10^{-5} and 2.58×10^{-4} respectively) strongly favour the solid phase, providing a basis for the design of a new method to enable better MIC measurement. A new method based on pre-mixing the volatile compound into the liquid media was developed. Testing showed that headspace volatile concentrations quickly stabilised and remained constant throughout the incubation period, making MIC determination easier.

The potential of each compound and their binary combinations to inhibit growth were evaluated using the new MIC measurement method. This resulted in very repeatable results with much lower headspace concentrations than measured using traditional methods. To test for synergistic effects in the multiple volatile trials, an alternative data analysis approach was adopted. The inhibition time before growth observed in each sample was linearized and regressed against the thymol and carvacrol concentrations. This resulted in a simple model with a significant thymol/carvacrol interaction term, clearly demonstrating a synergistic, although minor effect. The study showed the measurement of stable and repeatable MIC values for individual and combinations of volatiles is possible using the new method. These findings strengthen the possibility of using natural essential oils as alternatives to chemicals to preserve food products.

The key disadvantage of the new method is the requirement to mix the liquid essential oils directly into the liquid media before solidification. This prevents its application to solid food systems. For solid food systems, a system capable of delivering stable flows of air with volatiles at high concentrations in the presence of high relative humidity was designed. With this system, well-controlled and stable air compositions were achieved over two days, making the system suitable for measurement of the inhibitory effects on spoilage organism growth. Although further optimisation of the design and control of this system is required, it has the potential for collection of accurate target headspace conditions for controlled volatile release active packaging design.

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AI	Antifungal index
A_{GC}^{i}	Area of gas chromatogram peak from the injected volume of sample (Area).
CFU	Colony forming unit
C^{i}	Concentration of volatile organic compound (VOC) *i*, (mol.m^{-3})
C_{crv}	Carvacrol concentration (mol/m^{3})
DT	Detection time
EO	Essential oil
EVOH	Ethylene vinyl alcohol
FIC	Fractional inhibition concentration
Fh	Dry air flow rate for humidity (mL/min)
Fcrv	Dry air flow for carvacrol (mL/min)
FM	Mixture of dry air flow of carvacrol and humidity (mL/min)
GCMS	Gas chromatography–mass spectrometry
HDPE	High-density polyethylene
K_{GC}^{i}	Detector response or slope (mol. Area $^{-1}$) of a standard curve of VOC *i*.
LDPE	Low-density polyethylene
MAP	Modified atmosphere packaging
MFC1	Mass Flow Controller (mL/min) , humidity
MFC2	Mass Flow Controller (mL/min) , carvacrol liquid
MIC	Minimum inhibitory concentration
MLC	Minimum lethal concentration
OD	Optical density
OPP	Oriented Polypropylene

PD	Petri dish
PDA	Potato dextrose agar
PE	Polyethylene
PP	Polypropylene
ppm	Part per million
Psat	Saturated Vapour Pressure (Pa)
Pcrv	Partial Vapour Pressure for Carvacrol
P_h	Humidity Vapour Pressure (Pa)
$P_h/P_{h,sat\ (T2)}$	Humidity relative vapour pressure (Pa) at T_2°C
$P_{crc}/P_{crv,sat(T2)}$	Carvacrol relative vapour pressure (Pa) at T_2°C
RH	Relative humidity, (%)
RH_M	Relative humidity after mixture of carvacrol (%)
RH_2	Relative humidity at room temperature
Sp	Species
SPI	Soy protein isolate
T_1	Water bath temperature (°C)
T_2	Room temperature (25.0 ±1.0°C)
T_m	Temperature in mixing vessel (°C)
v/v	Volume per volume
Vol_{inj}	Injection volume of sample (m^3)
UV	Ultra-violet
w/w	Weight per weight