Design and evaluation of thermoplastic encapsulation and time-released diffusion of an antimicrobial fragrance preparation

For decades, various essential oils, for example Tea Tree Oil, have been known for their potential to be effective antimicrobials and have been used as such in topical treatments for various skin and scalp conditions, for example acne (caused by bacteria) and dandruff (caused by yeast)[1,2].
Further research has concluded that several other aroma chemicals also have potential antibacterial and antifungal properties[3,4].

Fresh Products has developed a method by which active antibacterial aroma chemicals, in liquid form, can be successfully encapsulated into thermoplastic co-polymer pellets (example, ethylene vinyl acetate, ethylene methyl acrylate) in high concentrations (>5% w/w) and produce a dry, free-flowing media. Further, Fresh Products has now demonstrated, via independent laboratory analysis, that the antibacterial aroma chemicals can be delivered in an efficacious manner via time-released passive vapor diffusion from the encapsulated co-polymer / fragrance media. Time-released passive vapor diffusion has shown to be an effective method to deliver antibacterial compounds and has the potential to be a preferred delivery mechanism to disperse effective antimicrobial compounds activity against odor-causing bacteria commonly found in sinks, drains, toilets and urinals.

Reference articles:

1. Clinical Microbiology Reviews 2006, Jan 19; Melaleuca alternifolia (Tea Tree) Oil: a review of Antimicrobial and Other Medicinal Properties; CF Carson, KA Hammer, TV Riley

2. International Journal of Dermatology 2013, July; A review of application of tea tree oil in dermatology; N Pazyar, R Yaghoobi, N Bagherani, A Kazerouni

3. International Journal of Aromatherapy 1998, Volume 8, Issue 4; Essential oils with high antimicrobial activity for therapeutic use; LR Williams, JK Stockley, W Yan; VN Horne

4. Medicines 2017, Sept 4; Antimicrobial Activity of Some Essential Oils – Present Status and Future Perspectives; S Chouhan, K Sharma, S Gueria

FINAL REPORT

PROTOCOL

AOAC 961.02
Modified Germicidal Spray Products as Disinfectants

EMSL ORDER NUMBER
242006694

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STUDY START DATE
October 13, 2020

STUDY COMPLETION DATE
October 30, 2020

Test Summary

Project Title: Custom protocol based on AOAAC 961.03 Germicidal Spray Method efficacy testing of EVA beads infused with fragrance.

Study Methods: Modified AOAC 961.02 Germicidal Spray Product as Disinfectant Method

Product Tested: EVA beads infused with fragrance EE20-45122 (from Premier Specialties)

Sponsor: Fresh Products, LLC.

Test Conditions:
Challenge Organisms:

1. *Escherichia coli* ATCC 8537
2. *Pseudomonas aeruginosa* ATCC 9027

Broth used: Modified Letheen Broth Neutralizing broth.

Contact time: 1 and 2 weeks.

Contact Temperature: Room temperature- 25 °C

Study Dates and Facilities
All analytical testing was performed at EMSL Analytical, Inc. in Wallingford, CT from 10/13/2020 to 10/29/2020.

Record Retention
All raw data and a copy of the final report will be archived and stored by EMSL Analytical, Inc. for 5 years.

Objectives
To determine the antimicrobial efficacy of the EVA beads infused with fragrance EE20-45122 (from Premier Specialties) against *E. coli, and P. aeruginosa* after 1, and 2 weeks exposure to the beads at room temperature.

Experimental Summary
The testing procedure was designed after discussions between EMSL Analytical Inc., the testing company, and the client, Fresh Products, LLC. The testing procedure is based on a modified AOAC 961.02 test method, with the testing conducted on EVA beads infused with fragrance (supplied by client), to demonstrate antibacterial efficacy against *E. coli and P. aeruginosa*.

Procedure:
Culture Preparation:
E. coli, and P. aeruginosa from stock culture were plated onto Tryptic Soy Agar and incubated at 35°C for 24 hours. After incubation a bacterial suspension was prepared for each of the bacteria by taking one 10µL loop of the test bacteria into 10 mL of Normal Saline 0.85% until a 10^8 solution of cells was created with initial concentrations as follows: *E. coli* with a 2.1 *x* 10^8 concentration, *and P. aeruginosa with a concentration of 81.4 x* 10^8.

Qualitative Test:

Sterile glass slide carriers were inoculated with 10 µL of each of the microorganism suspensions and incubated to air dry at 35°C for 40 minutes. Inoculated test carriers were individually placed in sterile vials containing 10 grams of EVA beads with fragrance and in sterile vials with untreated beads as controls. There were 3 carriers per treated beads, 3 carriers per untreated beads and viability control per organism tested per week.

The neutralized subculture of treated and untreated controls for *E. coli and P. aeruginosa* were incubated for 48 hours at 35.0°C. Viability controls of each bacterium were added to the corresponding subculture medium. A representative uninoculated carrier was added to the neutralizing subculture medium. All the subculture medium containing the carriers were visually examined for growth or no growth for all the bacterium types. The subculture medium containing the uninoculated carrier was incubated and examined for lack of growth.

Experimental Results:

Table 1. Control Results

Type of Control	Results	
	E. coli	*P. aeruginosa*
Purity Control	Pure	Pure
Viability Controls	Growth	Growth
Neutralizing Subculture Medium Sterility Control	No Growth	
Carrier Sterility Control	No Growth	

Table 2. Efficacy of EVA beads infused with fragrance EE20-45122 (from Premier Specialties)Test Results

Time Points (weeks)	Test Organism	Number of Carriers	
		Exposed	Showing Growth*
1	*Escherichia coli* ATCC 8537	3	0
	Pseudomonas aeruginosa ATCC 9027	3	0
2	*Escherichia coli* ATCC 8537	3	0
	Pseudomonas aeruginosa ATCC 9027	3	0

*Number of carrier showing growth of the test organism.

Conclusion:

Based on a Modified AOAC 961.02 test method, the EVA beads infused with fragrance EE20-45122 (from Premier Specialties) successfully demonstrated antibacterial efficacy against *E. coli* and *P. aeruginosa*.

Signature

Report Issued by:

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Microbiology Laboratory Director

11/09/2020
Date