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Short communication

Capsaicin migration through maple sap collection tubing

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Abstract

Capsaicins, present in most hot sauces and salsas, are responsible for the "hot" sensation in many spicy foods. At high doses, capsaicins cause significant discomfort upon contact with the sensitive tissues of the mouth and throat of mammals. By applying a capsaicin containing paste to the outside of maple tree sap collection tubing, operators hope to minimize rodent (primarily red squirrel, *Tamiasciurus hudsonicus*) gnawing damage to the tubing. However, some operators and sap processors have expressed concern regarding the potential migration of capsaicins through the tubing and into the tree sap, leading to contaminated maple syrup. To address these concerns, we filled a variety of new and used sap collection tubing with maple sap, plugged the ends, and coated the tubing with a commercially available capsaicin-based rodent repellent paste. Following storage, the contents of the tubes were carefully removed and subjected to a solid-phase extraction clean-up process. Capsaicins in the sap were then quantified by high performance liquid chromatography/fluorescence detection. Results indicate that polyethylene tubing was more resistant to capsaicins migration than was polyvinyl tubing. While capsaicins were detected in the sap, the predicted levels in syrup would be below the human taste threshold. Published by Elsevier Science Ltd.

Keywords: Squirrel; Maple syrup; Sap; Capsaicin, Repellent; Tubing

1. Introduction

In 1999, US maple syrup production was 1.2 million gallons. For each gallon of maple syrup produced, approximately 40 gallons of maple tree sap are collected and concentrated. Rodents cause losses and potential contamination of maple sap by chewing through the plastic tubing used to carry the maple tree sap to collection vessels. In Vermont, the state responsible for the largest maple syrup production, rodent damage to sap collection equipment is in excess of $300,000 annually (May and Slate, 1989). Historically, lethal techniques such as trapping, shooting, and rodenticide application, as well as non-lethal hazing and exclusion techniques, have been used to minimize these losses. All of these techniques have proven to be minimally effective or effective for a limited amount of time (May et al., 1992).

The repellent effect of capsaicins to various rodent species has been demonstrated (Wagner and Nolte, 2000). Capsaicins belong to a family of compounds known as capsaicinoids. These relatively lipophilic compounds consist of an aromatic moiety linked to an alkyl amide (Fig. 1). While capsaicins are responsible for the spicy "hot" taste of many foods, upon contact with mammalian tissues, capsaicins produce symptoms ranging from discomfort to mild pain (Haas et al., 1997; Christensen and Frank, 1996). It is this effect that is exploited in the development of capsaicin-based rodent repellents including an oleoresin of capsicum/petroleum jelly paste. By applying the capsaicin containing paste to the outside of the most vulnerable areas of the collection tubing system (generally the tubing nearest the tap trees), maple sap collectors hope to minimize rodent damage and associated losses. However, sap processors have expressed concern regarding the potential migration of capsaicins through the tubing and into the tree sap. Obviously, the potential consequences of capsaicin containing maple syrup are quite unsettling to the maple syrup industry and must be investigated before this technique can be widely adopted. The objectives of this research were to determine the potential for capsaicin migration through various types of tubing currently used to collect sap and to identify the types of sap

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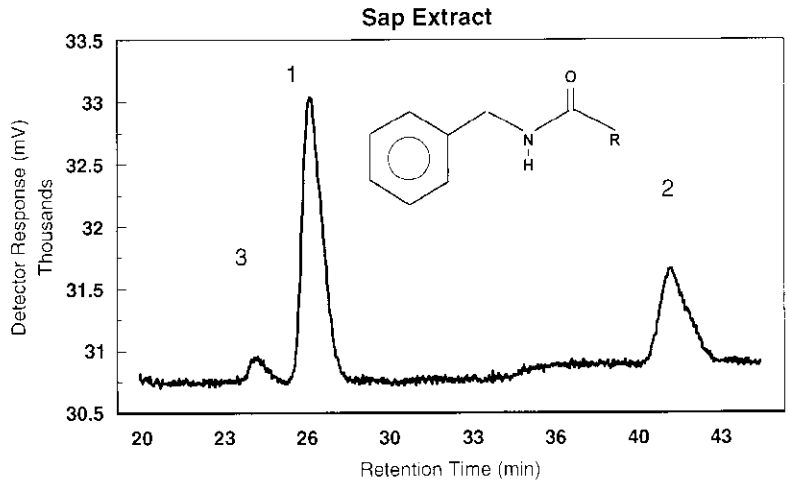

Fig. 1. Chromatogram of sap extract. 1 = capsaicin (R: $(CH_2)_4CH=CHCH(CH_3)_2$). 2 = dihydrocapsaicin (R: $(CH_2)_6CH(CH_3)_2$). 3 = nor-dihydrocapsaicin (R: $(CH_2)_5CH(CH_3)_2$).

collection tubing that can be safely coated with capsaicin-based rodent repellents.

2. Materials and methods

2.1. Chemicals

Tetrabutylammonium dihydrophosphate, potassium phosphate (for high performance liquid chromatography (HPLC) mobile phase) and natural capsaicin standard (mixed capsaicinoids) were purchased from Aldrich Chemical Co. (Milwaukee, WI). High purity methanol was obtained from EM Science (Gibbstown, NJ).

2.2. Test system

Samples of new and used sap collection tubing and maple tree sap were obtained from Wildlife Service cooperators in the New Hampshire/Vermont area. Based on consultations with industry experts, it was determined that the samples consisted of 1 new/13 used polyvinyl tubing samples and 3 new/4 used polyethylene tubing samples. In general, the polyvinyl tubing was more flexible than the polyethylene tubing. The tubing was cut into lengths such that the volume of the filled length of tubing would be approximately 40 ml. The tubes were washed with a mild soap solution and air dried prior to the start of the experiment. Each tube was sealed on one end by folding the tubing about 1 inch from the end and securing the folded section with a metal hose clamp. The tube was then filled with maple sap. The other end of the tube was sealed and the outside of the tube was coated with a commercially available formulation of oleoresin of capsicum in petroleum jelly. The 21 tubes were placed horizontally on a shelf in a dark room and left undisturbed for 90 days. At the end of the experiment, the capsaicin formulation was carefully removed from the outside of each tube by hand (latex gloves were worn to protect the skin). The formulation was placed in individually numbered glass jars for later analysis. The outside of each tube was subsequently washed with warm soapy water, rinsed with deionized water and then rinsed with acetone. The volume of each maple sap sample was then determined by pouring the contents of each tube into a 50-ml graduated cylinder. Capsaicin concentration of the sap was determined by HPLC/fluorescence detection. The entire experiment was repeated a second time.

2.3. Quantification of capsaicin in maple tree sap

The sap samples were transferred to 50-ml glass centrifuge tubes and centrifuged for 5 min at $1000 \times g$. The supernatant was eluted through an IST (Jones Chromatography, Lakewood, CO) solid phase extraction (SPE) cartridge containing 500-mg non-endcapped C-18 sorbent. The SPE cartridge was then washed with 3 ml of deionized water. Finally, capsaicins were recovered into a 15-ml glass centrifuge tube by elution with three 2.5 ml aliquots of methanol. This fraction was evaporated to near dryness under a gentle stream of nitrogen at 70°C. The residue was then reconstituted in 1 ml of methanol:water (1:1). This reconstituted capsaicin containing extract was briefly vortex mixed, sonicated for 10 min and centrifuged for 5 min at $1000 \times g$. The supernatant was transferred to an autosampler vial for analysis by HPLC. Capsaicins were quantified in this extract using the HPLC parameters listed in Table 1. The concentrations of capsaicin, dihydrocapsaicin and nor-dihydrocapsaicin in the sap samples were calculated

Table 1
HPLC parameters

Instrument	Hewlett-Packard 1090 HPLC		
Column	Keystone ODS, H (C18) 250 × 4.6 mm, 5 μm particle size		
Flow rate	1 ml min		
Mobile phase	Reservoir A: methanolic IPC A:aqueous IPC A (68:32) Reservoir B: methanolic IPC-A		
Solvent program	Minutes	% A	%B
	0.0	100	0
	15.0	100	0
	15.5	95	5
	50.0	95	5
	50.5	100	0
	55.0	100	0
Temperature	Ambient		
Injection volume	250 μl		
Detector	Spectrovision FD-300 fluorescence detector		
Detector voltage	800 V		
Detector response	1 s		
Detector range	500 nA		
Excitation wavelength	286 nm		
Emission wavelength	314 nm		

from a three-point linear regression curve (chromatographic response of external standards versus concentration).

To demonstrate the validity of this analytical approach for the quantification of capsaicins in maple tree sap, 14 replicates of sap were fortified with the natural capsaicin standard; seven were fortified at 0.0058 μg/ml (low level) and seven replicates were fortified at 0.2224 μg/ml (high level). These samples were then analyzed using the previously described methodology. The method limit of detection (MLOD) for each analyte was calculated as the concentration required to produce a chromatographic response three times greater than base line noise at the retention time for the analyte.

2.4. Statistical analyses

The total capsaicins concentration was calculated as the sum of the nor-dihydrocapsaicin, dihydrocapsaicin, and capsaicin concentrations in each sample. The mean and standard deviation of nor-dihydrocapsaicin, dihydrocapsaicin, capsaicin and total capsaicins were determined for each type of tubing using Microsoft Excel 2000 (Microsoft Corp., Redmond, WA). The significance of differences between capsaicin sap concentrations in different tubing types as well as between new and used tubing for each tubing type were determined by Analysis of Variance. Multiple comparisons were made using Least Significant Difference analysis (SAS/STAT, 1989).

3. Results and discussion

As demonstrated by the chromatogram in Fig. 1, the analytical method successfully isolated and separated the three major capsaicins from sap stored in tubing coated with a capsaicin-based rodent repellent formulation. The sensitivity of the analytical method to detect capsaicins in sap was nearly identical for each of the three major capsaicanoids (Table 2). The MLODs ranged from 0.00075 to 0.00078 μg/ml.

During method validation, the recoveries of the individual capsaicins ranged from 77 to 106 percent (Table 2). These results indicate that the analytical approach is acceptable for quantifying individual capsaicins in tree sap. For method validation, a natural product derived standard, containing the three major capsaicins, was used to fortify the tree sap. At the low fortification level, the concentration of nor-dihydrocapsaicin was below the method limit of detection. For this reason, recoveries for only dihydrocapsaicin and capsaicin are reported for the low fortification level.

All of the major capsaicins were detected in the sap stored in every type of tubing evaluated (Table 3). No

Table 2
Method limits of detection and method validation recoveries

	Nor-dihydrocapsaicin	Dihydrocapsaicin	Capsaicin
Method limits of detection (MLOD)			
Fortification level	0.00739 μg/ml	0.00122 μg/ml	0.00294 μg/ml
Peak height	154 mm	23.9 mm	62.7 mm
Baseline noise	5.3 mm	5.1 mm	5.3 mm
MLOD	0.00076 μg/ml	0.00078 μg/ml	0.00075 μg/ml
$n = 3$			
Method validation recoveries			
Low level			
Target (μg/mL)	0.0	0.00183	0.00398
Mean % recovery		93%	77%
Std. Dev.		13%	4.2%
CV		14%	5.5%
$n = 7$			
High level			
Target (μg/ml)	0.00894	0.0655	0.148
Mean % recovery	106%	97%	88%
Std. Dev.	8.1%	2.2%	2.3%
CV	7.6%	2.3%	2.6%
$n = 7$			

Table 3
Tubing type versus mean capsaicin concentration in sap (μg/ml)

	Nor-dihydrocapsaicin	Dihydro-capsaicin	Capsaicin	Total capsaicins
Polyvinyl 1				
Mean	0.0021	0.0486	0.02294	0.07345
Std. Dev	0.0016	0.1239	0.0501	0.1671
$n = 18$				
Polyvinyl 2				
Mean	0.0010	0.0028	0.0066	0.0103
Std. Dev	0.0011	0.0028	0.0078	0.01175
$n = 8$				
Polyvinyl 3				
Mean	0.0019	0.0107	0.0348	0.0474
Std. Dev	N/A*	N/A*	N/A*	N/A*
$n = 2$				
Polyethylene 1				
Mean	0.0002	0.0016	0.0005	0.0023
Std. Dev	0.0002	0.0019	0.0008	0.0028
$n = 4$				
Polyethylene 2				
Mean	0.0002	0.0018	0.0005	0.0024
Std. Dev	0.0002	0.0019	0.0005	0.0026
$n = 10$				

*Not applicable as sample size − 2.

significant differences were observed in capsaicin sap concentrations between new and used tubing ($\alpha = 0.05$). In general, the mean concentration of each compound was about an order of magnitude greater in the sap collected from the polyvinyl tubing as compared to the polyethylene tubing. This strongly suggests that the migration potential of capsaicins is greater when polyvinyl tubing is used to collect sap. The mean quantity of total capsaicins (0.0734 μg/ml) detected in the sap stored in polyvinyl tubing 1 was significantly greater than the other types of tubing ($\alpha = 0.05$). As sap is concentrated 40 fold to make syrup, this sap concentration would equate to a maximum syrup concentration of 2.94 μg/ml. For sap stored in polyethylene tubing, the highest mean total capsaicins concentration was 0.0024 μg/ml which would equate to a maximum concentration of 0.095 μg/ml in syrup. As the experiment was designed to represent a worst case scenario, static sap flow for 90 days, it is unlikely that these levels would be observed under field conditions. Additionally, since the human taste threshold for capsaicins is approximately 10 μg/ml (Merk Index, 1996), it appears that capsaicin-based repellents can be safely used to minimize rodent damage during sap collection. However, to add a margin of safety, it would be preferable to use polyethylene tubing rather than commonly used polyvinyl tubing.

References

Christensen, R., Frank, D., 1996. Preliminary investigation of oleoresin capsicum. National Institute of Justice Report 100-95. 10pp.

Haas, J., Whipple, R., Grant, P., Andersen, B., Volpoe, A., Pelkey, G.E., 1997. Chemical and elemental comparison of two formulations of oleoresin capsicum. Sci. Justice 37, 15–24.

May, A., Slate, D., 1989. An investigation of animal damage associated with maple syrup production. Proc. East. Wildl. Damage Control Conf. 4, 211–219.

May, A., Austin, J., Slate, D., 1992. Strategies to control rodent damage in sugar bushes: an update. Proc. East. Wildl. Damage Control Conf. 5, 187–191.

Merk Index, 1996. In: Budavari, S. (Ed.). An encyclopedia of chemicals, drugs and biologicals. Twelfth Edition. Merk & Co., Inc., Whitehouse Station, NJ.

SAS/STAT, 1989. User's Guide, Version 6, Vol. 2, Fourth Edition. SAS Institute, Inc., Gary, NC.

Wagner, K., Nolte, D., 2000. Evaluation of hot sauce as a repellent for forest mammals. Wildl. Soc. Bull. 28, 76–83.