Best Estimated Aroma and Taste Detection Threshold for Guaiacol in Water and Apple Juice

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ABSTRACT: *Alicyclobacillus acidoterrestris* can produce sufficient guaiacol (methoxyphenol), a metabolic by-product of the bacterium, in apple juice to cause a detectable taint characterized by an antiseptic off-odor or distinct medicinal flavor and lingering aftertaste. Bacterial spoilage may not be visibly detectable. The objective of this study was to determine the best estimate threshold (BET) for detection of guaiacol in water and commercial pasteurized apple juice from concentrate using the forced-choice ascending concentration method of limits with an experienced 17-member sensory panel. The mean BET for aroma detection of guaiacol in water and apple juice was 0.48 ppb and 0.91 ppb, respectively. The mean BET for taste detection of guaiacol in water and apple juice was 0.17 ppb and 0.24 ppb, respectively. Individual aroma BET values ranged from 0.06 ppb to 4.71 ppb guaiacol in water and 0.17 ppb to 4.71 ppb for guaiacol in apple juice. Individual taste BET values ranged from 0.01 ppb to 4.71 ppb for guaiacol in water and apple juice. The taste BET was equal to or lower than the aroma BET for guaiacol in both water and apple juice for all panelists. There was about a 500-fold range in guaiacol taste detection between panelists, with some individuals exhibiting a BET value as low as 10 ppt (trillion). The information should be useful for developing quality assurance sensory methodology to evaluate potential apple juice flavor spoilage by *Alicyclobacillus* spp.

Keywords: guaiacol, threshold, apple juice, *A. acidoterrestris*

Introduction

Over the past decade, some commercially pasteurized apple juice products that exhibited spoilage were characterized by a distinct off-flavor described as smoky, phenolic, and/or medicinal. The spoilage sometimes produced slight juice cloudiness, but in most cases, it was not visibly detectable (Walls and Chuyate 1998). The microorganism associated with the off-flavor was identified as *Alicyclobacillus acidoterrestris*, a thermoacidophilic, nonpathogenic spore former (Wisotzkey and others 1992). *A. acidoterrestris* spores are resistant to pasteurization, grow over a wide range of temperatures, and produce taint chemicals such as guaiacol (methoxyphenol) (Pettipher and others 1997), 2,6-dibromophenol, and 2,6-dichlorophenol (Jensen and Whitfield 2003). Guaiacol appears to be the predominant metabolite responsible for the described off-flavors in juice (Splittstoesser and others 1998).

Spoilage in juice products appears to be very slow and often unnoticeable until consumers complain about off-flavors. Spoilage happens in orange juice as well as other juices, but apple juice appears to be the most frequently spoiled juice product (Walls and Chuyate 2000). Studies on the sensory detection or recognition of guaiacol in juice products and, in particular, apple juice have been limited to aroma. Pettipher and others (1997) reported guaiacol levels up to 100 ppb in spoiled juice and an aroma threshold of about 2 ppb using an unknown sensory method. Orr and others (2000), when using an experienced sensory panel, determined the aroma best estimate threshold (BET) for the recognition of guaiacol added to uninoculated apple juice was 2.23 ppb. They also reported that the aroma sensory technique was more sensitive for detecting guaiacol in apple juice than the chromatographic method in which a solid-phase microextraction fiber cartridge was exposed to the test sample headspace and subsequently inserted in a splitless injector of a gas chromatograph coupled to a mass spectrometer as a detector in a selective ion-monitoring mode. Studies in our laboratory using the same or similar chromatographic techniques with microextraction fiber cartridges or headspace purge and trap sample concentrators have produced similar results. The chromatographic quantitation of guaiacol in apple juice was higher than the published aroma BET values.

When considering a method to screen apple juice samples for guaiacol that were potentially spoiled by *Alicyclobacillus* spp., sensory methodology appears to exhibit definite advantages over analytical methodology in terms of sensitivity, the ability to screen many samples fast, and in equipment cost. Sensory techniques using taste rather than aroma may further lower the sensitivity threshold for guaiacol detection in apple juice. Therefore, the purpose of this study was to determine the taste detection threshold of guaiacol in commercial apple juice expressed as a BET value and to determine the sensitivity range of the pre-screened experienced panelists. The aroma BET value for guaiacol in apple juice was also determined to compare with published values and with the taste BET value. Because apple juice may consist of a single variety or a varietal blend, the intensity and type of apple flavor may potentially suppress the aroma and taste BET values. To examine the potential impact, water was chosen as a comparative medium, and the taste and aroma BET values were determined.

Materials and Methods

Procedure for detection threshold determination

The aroma and taste detection thresholds for guaiacol in water and in commercial apple juice were determined by using the forced-choice ascending concentration method of limits described by the American Society for Testing and Materials, designation E679-91 (ASTM 2003), and similar to that described by Orr and

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others (2000). Commercial pasteurized apple juice made from concentrate (Tree Top Inc., Selah, Wash., U.S.A.) and 18 megohm (reverse osmosis/deionization (RO/DI) water was supplemented with guaiacol (Sigma, St. Louis, Mo., U.S.A.) concentrations of 0, 0.01, 0.03, 0.10, 0.30, 0.91, 2.72, 8.15, 24.4, and 73.3 ppb. Each panelist was presented with a cup set consisting of 3 coded 104-mL plastic odor free cups (Solo nr P35, Solo Cup Co., Urbana, Ill., U.S.A.) containing about 30 mL of liquid served at 21 ± 1 °C. One cup in each set contained guaiacol. Cups within each set were presented to panelists in random order. Panelists were instructed to smell or taste the cups in order from left to right and to decide which sample was different from the other 2. A choice was required even if they could discern no difference. For each of the threshold determinations, panelists were given cup sets starting with 0 ppb guaiacol followed by successive cup sets containing increased guaiacol concentrations. Evaluations continued until the cup containing guaiacol in 2 consecutive cup sets was correctly identified. Each aroma and taste threshold determination procedure for guaiacol in water and in commercial apple juice was repeated 3 times. All evaluations were conducted in a sensory panel room containing 4 side-by-side separated booths temperature controlled at 21 ± 1 °C.

Panelists

The panel consisted of 17 individuals who were aroma and taste prescreened using a guaiacol concentration of 73.3 ppb in water. Because all the panelists were trained to recognize guaiacol aroma and taste, the lowest recognized stimulus would be classified as the "detection" threshold according to ASTM E679-91 (ASTM 2003).

Calculations

The panel BET detection value for guaiacol aroma and taste in water and in commercial apple juice was calculated by combining the average geometrical means of the BET of the 3 replicates for each panelist and for each individual panelist replication. BET detection values were also calculated for each panelist for each replication to determine the overall panel sensitivity range. The individual BET ranges for each medium were listed in Table 1.

Results and Discussion

The calculated guaiacol BET detection values for each of the 3 replicates and the combined replicates (overall) for aroma and taste in water and in apple juice are shown in Figure 1. The mean BET aroma detection values for guaiacol in water and apple juice were 0.48 ppb and 0.91 ppb (Figure 1), respectively. The mean BET taste detection values for guaiacol in water and apple juice were 0.17 ppb and 0.24 ppb (Figure 1), respectively. In both mediums, the BET taste detection values were about 2-fold to 3-fold lower than the corresponding BET aroma detection values (Table 1). Taste methodology appears to be more sensitive than aroma methodology for detecting guaiacol in apple juice.

When comparing apple juice to water as a medium, the guaiacol BET values were slightly lower in water for both aroma and taste (Table 1). The taste BET range values for both mediums were the same, suggesting that the apple flavor did not suppress guaiacol detection in apple juice for many of the panelists.

Individual BET aroma detection values ranged from 0.06 ppb to 4.71 ppb for guaiacol in water and 0.17 ppb to 4.71 ppb for guaiacol in apple juice (Table 1). Individual BET taste detection values ranged from 0.01 ppb to 4.71 ppb for guaiacol in water and in apple juice resulting in an approximate 500-fold range between panelists within a panel (Figure 1).

These results for BET aroma detection values are similar to, but slightly lower than, those reported by Orr and others (2000) at 2.23 ppb and Pettipher and others (1997) at about 2 ppb (Table 1). The lower detection values in this study can probably be attributed to the fact that the panelists were pre-trained to recognize guaiacol aroma and taste and were instructed to detect differences between samples. This is the 1st known study in which BET taste detection values have been reported for guaiacol in water and apple juice.

Table 1—Comparison of best estimate threshold (BET) (ppb) values and panelist BET range values between published studies and this study

Medium	BET (ppb)	Panelist BET range (ppb)
Aroma – Water	0.48	0.06 to 4.71
Aroma–apple juice	0.91	0.17 to 4.71
Aroma–apple juice (Orr and others1997)	2.23	1.84 to 2.92[a]
Aroma–juice (Pettipher and others 1997)	About 2[b]	—
Taste–water	0.17	0.01 to 4.71
Taste–apple juice	0.24	0.01 to 4.71

[a]Defined as "detection recognition range."
[b]Different sensory method.

Conclusions

This study and others (Pettipher and others 1997; Orr and others 2000) indicate that the aroma BET detection value for guaiacol in apple juice was within the range of 1 to 2 ppb. Based on the results in this study, the taste BET detection value for guaiacol in apple juice appears to be near 0.24 ppb. In general, taste methodology appears to be about 4-fold to 5-fold more sensitive for evaluating potential guaiacol spoilage in apple juice than aroma methodology.

There was very little difference between the guaiacol taste BET values between the 2 mediums, water and apple juice, suggesting that water may be a good alternate medium to use for panel training. There was an apparent wide range of sensitivities between individuals that have the ability to taste guaiacol by as much as about 500-fold. When using sensory techniques to evaluation potential apple juice spoilage by *Alicyclobacillus* spp., use of taste method-

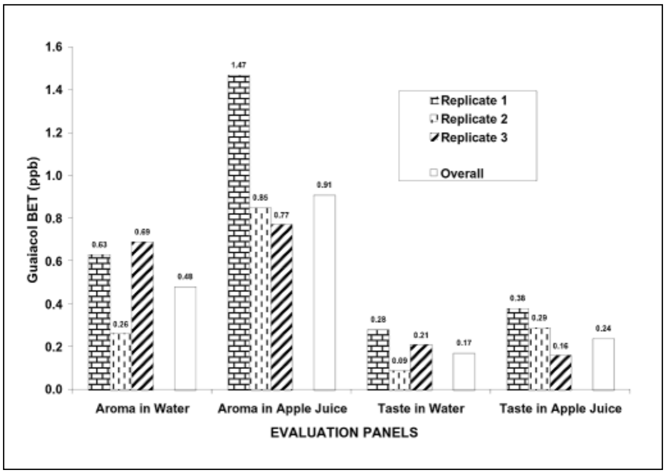

Figure 1—The guaiacol calculated best estimate threshold (BET) values for each of the 3 panel replicates and the overall value are shown for aroma and taste in water and apple juice.

ology and screened panelists with the greatest sensitivity to guaiacol should be considered.

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