DETERMINATION OF ORTHO-NASAL AROMA THRESHOLD FOR MULTICOLORED ASIAN LADY BEETLE IN A CONCORD GRAPE JUICE

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ABSTRACT

Sensory evaluation was used to determine the aroma threshold of Harmonia axyridis *(multicolored Asian lady beetle [MALB]) in Concord grape juice. Prior to Concord grape juice preparation, MALB was added to the Concord grapes at concentrations of 0 and 8.4 MALB/kg fresh grapes. To determine the odor threshold, the three alternative forced-choice method of sample presentation was used, with an ascending concentration series of 0.5, 1.5 and 4.5 MALB/kg fresh grapes. The panel aroma threshold concentration, calculated using the best estimate threshold over two panel sessions, was 1.8 MALB/kg fresh grapes. Individual detection thresholds ranged from 0.29 to 7.79 MALB/kg fresh grapes, with 75% of the panelists having a detection threshold at or below 0.87 MALB/kg fresh grapes. These results indicate the influence of low numbers of MALB on Concord grape juice aroma, and suggest the importance of quality control programs, including the implementation of established tolerance limits, to monitor grape quality.*

PRACTICAL APPLICATIONS

Harmonia axyridis, the multicolored Asian Ladybeetle (MALB), was originally introduced as a method of biocontrol. However, its presence is now becoming problematic with respect to crop quality. In order to minimize economic losses to the Concord grape industry, determination of the number of MALB required to produce a detectable change in Concord grape juice is

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critical for juice producers in the establishment of their quality control programs. Therefore, the objective of this study was to determine the aroma threshold level of MALB in Concord grape juice. The panel aroma threshold concentration, calculated using the best estimate threshold was 1.8 MALB/kg fresh grapes, with 75% of the panelists having a detection threshold at or below 0.87 MALB/kg fresh grapes. These results indicate the influence of low numbers of MALB on Concord grape juice aroma, and suggest the importance of quality control programs, including the implementation of established tolerance limits, to monitor grape quality.

INTRODUCTION

The multicolored Asian lady beetle (*Harmonia axyridis*: Coleoptera: Coccinellidae [MALB]) was originally introduced in the U.S.A. as a method of biocontrol for aphids and other soft-bodied insects (Nalepa *et al.* 1996). In the southeastern U.S.A., the first established population of MALB was recorded in 1988, and in successive years, the MALB population has continued to spread north (Chapin and Brou 1991). Currently, MALBs are widespread throughout northeastern U.S.A. and eastern Canada, with some reports in the western U.S.A. (Cattell 2002). In grapes, the establishment of MALB has had both beneficial and detrimental consequences (Koch *et al.* 2004). As beneficial insects, MALBs have served as biocontrol agents. However, the presence of MALB is now becoming problematic with respect to crop quality as adult MALB may be incorporated into harvested fruits. Reports have noted MALBs present in ripening grape clusters, with 20–50 MALBs reported on some clusters (Martinson 2002).

Under stress, MALBs produce a range of chemical compounds as a defensive mechanism. These compounds are of olfactory significance to humans, and have been associated with objectionable odors (Rothschild and Moore 1987). In grapes, the presence of MALB in the grape clusters and subsequent inclusion into the processing sequence may result in the release of these compounds and a final product with unacceptable flavors and aromas (Ker and Pickering 2004). The compound attributed to conferring these unacceptable aromas has been identified as 2-isopropyl-3-methoxypyrazine (IPMP; Pickering *et al.* 2004b). This compound, secreted by the MALB, serves dual roles, both as a pheromonal attractant between adult MALBs, and as an alerting signal to potential predators (Al Abassi *et al.* 1998). IPMP was found to have a low odor detection threshold and has also been reported to impart off-odors to wines (Pickering *et al.* 2005). Methoxypyrazine has been reported to have a detection threshold of 2 ng/L in water (Seifert *et al.* 1970), 4–8 ng/L in white wine and 8–15 ng/L in red wine (Allen and Lacey 1999).

A recent study has examined the impact of MALB taint on the sensory properties of red and white wine and has demonstrated the potential of MALB to influence final wine quality. In that study, *H. axyridis* (MALB) was added to white grape wine musts at a rate of 0, 1 or 10 MALB/L, and resulting wines were analyzed using a trained sensory panel (Pickering *et al.* 2004b). Results indicate the impact of MALB addition on the quality of wine at concentrations as low as 1 MALB/L.

The impact of MALB on Concord grape (*Vitis labruscana* Bailey) juice quality has not been examined. Over the past 2 years, Concord grape production in the U.S.A. was reported as 563, 760 tons (2005) and 372, 870 tons (2006). Based on tonnage, Washington State is the major production state for Concord grapes in the U.S.A., growing 47% of the Concord crop in 2006 (USDA 2007). In order to minimize economic losses to the Concord grape industry, determination of the number of MALB required to produce a detectable change in grape juice is critical for juice producers in the establishment of their quality control programs. Therefore, the objective of this study was to determine the aroma threshold level of MALB in Concord grape juice.

MATERIALS AND METHODS

Concord Grapes and MALB

MALBs were field collected from cornfields in Yakima Valley, WA. Live MALBs were maintained over ice prior to addition to the grape juice. Concord grapes were harvested from Irrigated Agriculture Research and Extension Center, Washington State University (Prosser, WA) at commercial maturity.

Preparation of Concord Grape Juice

Grape juice was prepared in triplicate with (treated) and without (control) the addition of MALB. The MALB concentration was decided upon as a high concentration that could be diluted for sensory evaluation studies. For treated juice preparation, 800 MALBs were counted and added to grapes (95 kg) prior to juice preparation. As the live MALBs were maintained on ice, no losses in MALBs were observed during processing. To prepare grape juice, grapes were stemmed, weighed and transferred to a steam-jacketed kettle, with agitator, and heated to 60C. Enzyme (Scottzyme Pec 5 L (Scott Laboratories, Petaluma, CA), 0.75% w/w) was added to the grape mash and held for 35 min to complete enzyme treatment. The grape mash was pressed in a screw press. The resulting juice was pasteurized at 85C for 2 min, after which the juice was immediately cooled to 21C and allowed to settle at 4C. After 1 week, juice was transferred to 1-L glass canning jars and boiling water bath was pasteurized for

10 min and then cooled to 21C. The final yield from the Concord grapes was 113 L of grape juice. The final concentration of treated juice was 8.4 MALB/kg of grapes. This juice was diluted to prepare the appropriate concentrations for threshold testing.

Sensory Analysis

The sensory panel was recruited from Washington State University, and 18 untrained panelists between the ages of 18 and 65 participated in each sensory panel. The panelists were screened for known anosmias and other conditions such as the common cold, which might affect their performance in this panel. A minimum amount of information on the nature of the study was provided in order to reduce potential bias and expectation error; the panelists were only informed that they were assessing Concord grape juice.

Each panel was composed of 10 females and 8 males. Replicate sensory panels were run on different days; thus, the panelists who participated in each panel might have been different. Sensory threshold for ortho-nasal olfaction was determined using the forced-choice ascending concentration series triangle test (3-AFC), as described by American Society for Testing and Materials (ASTM) (ASTM E 679-04 (2004). Ortho-nasal detection was selected as the panelists have been found to have a low aroma threshold for methoxypyrazines (Allen *et al.* 1991), and carryover between samples was a concern. Three scale steps were used, with a threefold dilution factor between steps. Control juice (juice not containing any MALB) was used to dilute the MALB juice and make up the concentration series. Three scale steps were selected because of the large carry-over effect between samples and the desire to limit this effect. At each selected concentration, a 3-AFC sample set was prepared, consisting of one sample and two reference (blank) samples. The samples (30 mL) were served to each panelist as a series of three blind-coded sets of three samples per flight. The flight containing the lowest concentration of MALB was presented first, followed by increasing concentrations. The panelists indicated the sample that they identified as different from the other two in each flight. As the method employed forced choice, the panelists were required to guess if they could not discriminate.

Preliminary panels indicated no sensory differences ($P > 0.05$) between replicate grape juice batches; therefore, batches were pooled. Preliminary studies were also used to select the appropriate scale-step range of MALB concentrations employed in the threshold test. Two preliminary studies were conducted. The first study used concentrations of 7.5, 10.5, 15.0 and 21.0 MALB/kg grapes; results from this study indicated that the majority of panelists were correct at all concentrations. A second preliminary study was

conducted whereby concentrations of 0.004, 0.031, 0.25 and 2.0 MALB/kg grapes were studied, and the results from this study showed the majority of panelists were incorrect at the different concentrations. Based on the results from these preliminary sensory panels, the concentrations of MALB in grape juice used in the final aroma threshold determination studies were 0.5, 1.5 and 4.5 MALB/kg grapes. Red lights were used to mask potential differences in grape juice color.

Data were analyzed and the best estimate threshold (BET) was calculated using the procedure described by ASTM E 679-04 (2004). The individual BET was determined as the geometric mean of the highest concentration missed and the next concentration. For those panelists who were correct at the lowest MALB concentration, their individual BET was estimated as the geometric mean of the lowest MALB and the hypothetical next lower MALB concentration that would have been given had the series been extended. For those panelists who failed to correctly identify the MALB grape juice at the highest MALB concentration, their individual BET was estimated as the geometric mean of the highest MALB concentration tested in the study and the next higher MALB concentration that would have been given had the series been extended. The group BET was calculated at the geometric mean of the individual BET. Differences between two replicate panels were calculated using Student's *t*-test in Excel.

RESULTS

The MALB concentrations in the ascending series of grape juice were 0.5, 1.5, and 4.5 MALB/kg grapes. The individual BET expressed as MALB/L grape juice for each panel session are shown in Fig. 1. The panel aroma threshold concentration, calculated using the BET, was determined to be 1.8 MALB/kg fresh grapes over the two panel sessions. While a difference in BET between the two sessions was observed, this difference was not significant ($P \geq 0.05$). While in this study the first panel showed a lower BET than in the second panel, it is more common to observe a replicate panel with a lower BET as the panelists become more familiar with the task and specific sensory cues differentiating the samples.

The range of detection thresholds is shown as the cumulative frequency distribution of individual best estimate detection threshold for detection of MALB taint in grape juice (Fig. 2). The detection thresholds ranged from 0.29 to 7.79 MALB/kg grapes, with 75% of the panelists having a detection threshold at or below 0.87 MALB/kg fresh grapes. The samples had a large enough range of MALB/kg fresh grapes so that on average between the two sessions, 86% of the panelists correctly identified the odd sample twice in a row, when presented as increasing concentrations.

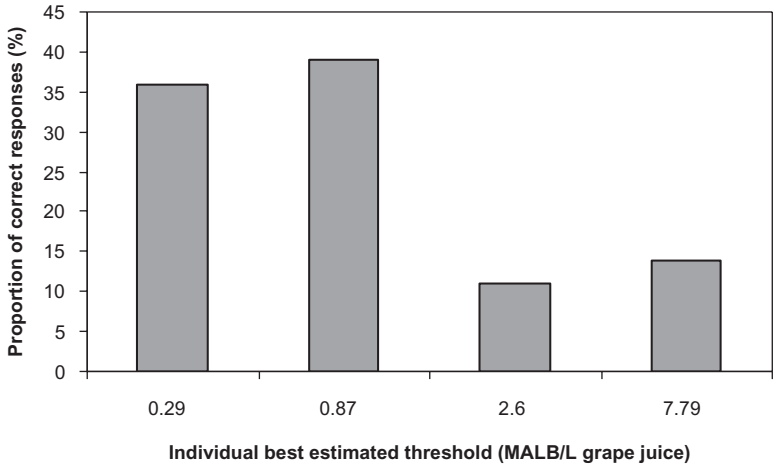

FIG. 2. CUMULATIVE FREQUENCY DISTRIBUTION OF INDIVIDUAL BEST ESTIMATE DETECTION THRESHOLD FOR DETECTION OF MULTICOLORED ASIAN LADY BEETLE (MALB) TAINT IN GRAPE JUICE
Data from the two panel sessions were combined.

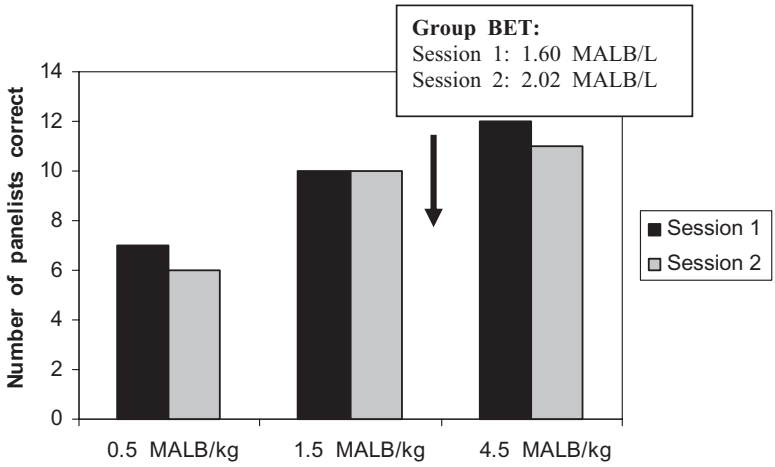

FIG. 1. NUMBER OF PANELISTS CORRECT AT EACH CONCENTRATION STEP, 0.5, 1.5 AND 4.5 MALB/L, OVER TWO REPLICATE SESSIONS
Individual best estimate threshold (BET) expressed as MALB per liter grape juice for each panel session.
MALB, multicolored Asian lady beetle.

DISCUSSION

The presence of MALB was found to influence the aroma of grape juice at a presence of 1.8 MALB/kg fresh grapes. This aroma threshold was similar to the results reported by Pickering *et al.* (2004b). In white and red wine, Pickering *et al.* (2004b) examined the influence of MALB at two levels, 1 and 10 MALB/kg grapes, and found significant modifications in the sensory properties of the wine were observed at concentrations of 10 MALB/L wine, with observable modifications at 1 MALB/L. In red wine, the presence of MALB resulted in significantly higher ratings for asparagus or bell pepper, peanut and earthy herbaceous notes in both aromas and flavors, while in white wines, higher ratings were assigned to peanut, bell pepper and asparagus notes. Anecdotally, the profile of MALB-tainted grape juice was different from the aroma profile of tainted wine. The aroma of the MALB-tainted grape juice was described as more musty and dirty, when compared with the control grape juice. Further research is being pursued examining the specific impact of MALB taint on Concord grape juice, consumer acceptance of tainted Concord grape juice and the role of the Concord grape juice's high aroma intensity in moderating the effects of MALB.

Based on the previous studies of MALB taint in wine, preliminary determinations of the appropriate scale-step range of MALB for threshold studies used 21 MALB/kg fresh grapes as the highest concentration in the flight. The difference in processing between the wine and grape juice was considered in the determination of the original series of threshold concentrations. Because of the strong odor of Concord grape juice, it was believed that a high concentration of MALB would be required to compete with the Concord aroma and would result in a sensory difference. Through subsequent testing, this was not found to be the case, resulting in the final threshold testing range selection of 0.5–4.5 MALB/kg fresh grapes. The presence of ethanol in the wine and the polarity of methoxypyrazine were also considered as major factors in the determination of the threshold range.

CONCLUSION

These results indicate the influence of low numbers of MALB on Concord grape juice aroma. The low concentration of MALB required to impact the character of grape juice suggests the need to establish MALB tolerance limits in quality control programs in order to monitor grape quality.

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