THE IMPACT OF MULTICOLOURED ASIAN LADY BEETLES ON THE SENSORY PROPERTIES OF CONCORD AND NIAGARA GRAPE JUICE

by

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The members of the Committee appointed to examine the thesis of LUAN WEEKES find it satisfactory and recommend that it be accepted.

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THE IMPACT OF MULTICOLOURED ASIAN LADY BEETLES ON THE SENSORY PROPERTIES OF CONCORD AND NIAGARA GRAPE JUICE

ABSTRACT

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The presence of Multicoloured Asian Lady Beetles (MALB) in juice and wine can cause objectionable aromas and flavours. The overall hypothesis of this study was that the presence of MALB can significantly affect the aroma, taste/flavour and mouthfeel properties of Concord and Niagara grape juice. The first objective was to determine if MALB changed the analytical values of the juice. For Concord juice, significant decreases were observed with pH, Brix and titratable acidity. For Niagara juice, pH significantly decreased and titratable acidity significantly increased with increased MALB taint. The second objective was to determine the best estimate threshold (BET) of MALB in both juices. Panelists (n= 48) determined the BET for Concord grape juice was 1.98 MALB/L and 0.65 MALB/L for Niagara grape juice. Following storage of Concord grape juice for 11 mo, panelists (n=17) determined the BET was 1.66 MALB/L. The third objective of this study was to determine which sensory characteristics of the grape juices were significantly impacted by the presence of MALB. Ten trained panelists (n=10) found significant differences in Concord grape juice in vegetal aroma and earthy flavour as MALB increased from 0.45 MALB/L to 7.2 MALB/L. For Niagara grape juice, the trained panel (n= 8) found significant differences in honey, vegetal and earthy aromas and flavours, grape flavour, sweetness, sourness and astringency. The fourth objective of the study was to determine which juice, MALB or control was preferred by consumers (n=60). For Concord grape juice, consumers significantly rejected the aroma of MALB juice at 1.8 and 3.6 MALB/L. For taste/flavour, significant rejection of MALB juice occurred at 3.6 MALB/L. For Niagara juice, consumers significantly rejected the aroma of the juice at 3.6 MALB/L. This study demonstrated the presence of MALB can negatively impact the sensory properties of Concord and Niagara grape juice. More research is needed to determine the remediation of grape juice should MALB taint occur. Grape juice processors can use this research for quality control measures and for setting tolerance limits.

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CHAPTER ONE

INTRODUCTION

The United States is one of the world's largest producers of grapes and is the largest user of grape juice and grape juice concentrate (Pollack et al. 1997; Bates et al. 2001). Ninety percent of the grape crop in the US is produced in California, followed closely by New York and Washington state. Grapes grown in New York and Washington are mainly used for juice and wine production. In the United States, trends for consumption of processed grape products vary. During the 1990s, grape juice consumption rose from 2.5 to 4.1 pounds per person (Pollack et al. 1997). Although the grape juice industry is a small part of the overall fruit juice industry, the consumption of grape juice has increased since 1977 at a rate of 2.2% per year. Concord blends have become the standard for red grape juice around the world. For white grape juice, Niagara along with Delaware and Catawba and various labrusca blends are gaining popularity (Amanor-Boadu et al. 2003).

One issue becoming a problem with grapes is the presence of the multicoloured Asian Lady beetle (MALB). *Harmonia axyridis* Pallas (Cleoptera:Coccinellidae) is a predator coccinellid, native to central and eastern Asia. It feeds on a range of soft bodied insects such as aphids, ladybeetles and mites and is commonly found in grain, soybean, conifers and fruit crops in the Americas and Europe. MALB are also found on crops such as pecans, apples, citrus and corn (Kenis et al. 2008; Koch 2003; Galvan 2008). Despite the documented release of *H. axyridis* in North America, it is argued that these early MALB populations resulted from unintentional introductions via international commerce rather than from the documented intentional releases for biological control (Day et al. 1994). For the purpose of biological control, introductions of the species were made in

North America between 1916 and 1985, with introduction in California in 1916, 1964 and 1965 (Gordon 1985; Soares et al. 2007). Since the 1980s, the population of MALB has spread and increased dramatically to become the dominant ladybeetle species in the USA and Canada.

MALB vary in colour and patterns. The adults are oval, convex and about 6 mm long and 5 mm wide. The North American populations have a variety of colours ranging from pale yellow-orange to bright red-orange with or without black spots on the wing covers. The head, antennae and mouthparts are generally straw-yellow but they are sometimes tinged with black. The protonum or back is also straw-yellow and can vary in spots from many to few to none. The most commonly identifying feature located on the protonum is a black "M" inscribed just preceding the wing covers (elytra), which is consistent with most MALB adults (Ker et al. 2002; Baniecki et al. 2004).

MALB can be a household nuisance and a fruit production pest mainly in late autumn until early spring hibernating in cracks and crevices. In Ohio, October 1993, some residents reported that thousands of MALB were congregating on homes and buildings and also finding their way indoors (Jones et al. 2002). Apart from being a household nuisance, MALB can be a pest of fruit production, especially as a contaminant in wine production (Pickering et al. 2004; Galvan et al. 2006). MALB are attracted to ripening grapes as a source of sugar late in the season. MALB search for sugar-containing food sources prior to moving to aggregation sites. This may be the main reason for being attracted to damaged wine grapes because adult MALB are not able to directly break the grape berry skins (Galvan et al. 2008). Depending on the number or clusters damaged, MALB can easily infest up to 65% of all clusters for some grape cultivars (Galvan et al. 2006b; 2008). When MALB are crushed along with the grapes, the compounds released cause the wine to smell "dirty" and mask some of the wine characteristics (Anonymous 2005). The MALB problem occurs in cultivars such as Cabernet Franc, Cabernet Sauvignon, Riesling, Vidal and Vignoles (Anonymous 2005). Ross et al. (2007) researched the sensory evaluation of Washington State Merlot suspected of MALB taint using untrained panelists. The MALB tainted wine had more musty/earthy and vegetal aroma, green pepper flavour and sour taste while the control had a more floral/fruity aroma. Panelists preferred the control wine over the MALB tainted wine. These results were similar to Pickering et al. (2004) who researched the influence of MALB on the sensory properties of red and white wine using trained panelists.

When crushed or disturbed, MALB engage in "reflex bleeding" and release a yellow fluid called hemolymph. The hemolymph contains alkaloids and pyrazines (Galvan et al. 2008). These compounds can affect the odour, taste and flavour of the wine resulting in the complete loss of the contaminated wine and/or increased production costs from additional time and labor need to control MALB (Galvan et al. 2008; Galvan et al. 2007; Pickering et al. 2006a). The MALB problem occurs mainly due to its presence on fruit clusters or containers upon delivery and inclusion in the processing sequence (Ker et al. 2006). Pyrazines are identified as the contaminant contributing to MALB taint (Pickering et al. 2007). Pyrazines contribute green bell pepper, beans and asparagus aromas as well as green, earthy, herbaceous, vegetative and bell-pepper like attributes (Boubee et al. 2000; Pickering et al. 2007). The human olfactory threshold for methoxypyrazine in water is two parts per trillion (Cai et al. 2007). Pickering et al. (2007)

investigated the detection of 2-isopropyl-3-methoxypypyrazine in red and white wine by untrained panelists. The orthonasal and retronasal threshold for red wine blend is 1.03 parts per trillion and 2.29 parts per trillion respectively. For Gewürztraminer, the orthonasal and retronasal threshold is 1.56 parts per trillion and 1.15 parts per trillion, respectively. For Chardonnay, the orthonasal threshold is 0.32 parts per trillion. Pickering et al. (2008) investigated the detection thresholds of 2- isopropyl-3-methoxypyrazine in Concord and Niagara grape juice. The orthonasal and retronasal threshold for Concord grape juice is 1.11 parts per trillion and 1.02 parts per trillion respectively, while for Niagara grape juice it is 0.74 parts per trillion and 0.84 parts per trillion. Ross et al. (2007) added MALB to Concord grape juice at rates of 0.5, 1.5 and 4.5 MALB/kg and the orthonasal threshold was 1.8 MALB/kg. Galvan et al. (2007) also added MALB ranging from 0.3 to 8.0 MALB/kg to wine made from Frontenac winegrapes and the orthonasal threshold is 1.9 MALB/kg. However, despite the low threshold of MALB, the presence of MALB in juice and wine impacts the resulting sensory properties. Pickering et al. (2004) found the presence of MALB at 1 and 10 MALB/L increased peanut, bell pepper and asparagus aroma and flavours in white wines and increased earthy/herbaceous, asparagus/bell pepper aroma and flavours in red wines. Floral and fruity intensities decreased in both red and white wine.

Research has shown the presence of MALB can influence the sensory properties of red and white wine. The objective of this study was to determine if MALB has a significant impact on the sensory properties of Concord and Niagara grape juice. Analytical measures such as titratable acidity (TA), Brix and pH were also determined to give an indication of contamination. It was hypothesized that MALB-tainted grape juice had a low aroma threshold, possessed sensory characteristics that make it easily distinguishable from untainted MALB juice and has a low consumer rejection threshold. It was also hypothesized MALB in Niagara gape juice would be more easily recognizable than in Concord grape juice. The recognition of MALB taint characteristics would also allow grape juice processers to identify tainted juices. Results of this study can be used in quality control and in setting tolerance limits of MALB in grape juice.

CHAPTER TWO

LITERATURE REVIEW

History and description of beetle

The multicoloured Asian Lady beetle (MALB), *Harmonia axyridis* Pallas (Cleoptera:Coccinellidae), is a predator coccinellid, native to central and eastern Asia. It feeds on a range of soft bodied insects such as aphids, ladybeetles and mites and is commonly found in grain, soybean, conifers and fruit crops in the Americas and Europe, as well as in greenhouses. MALB are also found in crops such as pecans, apples, citrus and corn (Kenis et al. 2008; Koch 2003; Galvan 2008).

Despite the documented release of *H. axyridis* in North America, it has been argued that these early populations resulted from unintentional introductions via international commerce rather than from the documented intentional releases for biological control (Day et al. 1994). Introductions of the species were made in North America between 1916 and 1985, and mainly in California in 1916, 1964 and 1965 (Gordon 1985; Soares et al. 2007) for the control of pecan aphids. Since the 1980s, the population of MALB has spread and increased dramatically to become the dominant ladybeetle species in the USA and Canada.

MALB has over 100 forms of various colours and patterns. The adults are oval, convex and about 6 mm long and 5 mm wide. The North American populations have a variety of colours ranging from pale yellow-orange to bright red-orange with or without black spots on the wing covers. The head, antennae and mouthparts are generally straw-yellow but they are sometimes tinged with black. The protonum or back is also straw-yellow and can vary in spots from many to few to none. The most commonly identifying feature located on the protonum is a black “M” inscribed just preceding the wing covers (elytra) which is consistent with most MALB adults (Ker et al. 2002; Baniecki et al. 2004).MALB can be a household nuisance and a fruit production pest mainly in late autumn until early spring. In their native habitat, large aggregations of MALB often hibernate (overwinter) in cracks and crevices within cliff faces. Overwintering may be trigged by changes in temperature, food resources and reproductive capacity. MALB can survive freezing temperatures and are classified as freeze intolerant. Freeze intolerant insects can avoid ice formation by supercooling. Supercooling is the temperature at which body fluids spontaneously freeze when cooled below the melting point (Zachariassen 1985). MALB become acclimated for winter by decreasing their supercooling point and lowering their lethal temperature, resulting in an increase in the levels of myo-inositol, a cryoprotective agent (Wantanabe 2002; Koch et al., 2004b; Galvan et al., 2008).

In the United States, MALB seek overwintering sites in and around buildings. In Ohio, in October 1993 some residents reported that thousands of lady beetles were congregating on homes and buildings with many of these insects finding their way indoors (Jones et al., 2002). An article in the New York Times (2005) showed many homeowners in Kentucky, Ohio and West Virginia desperately wanting to be rid of the insects. One Kentucky resident stated they were falling into food and drinks. Winemakers in Ontario, Canada recalled some vintage 2001 wine which has a ladybeetle bouquet. High populations of MALB are reported in Washington State, specifically Chelan, Klickitat and Yakima counties and in Oregon, Georgia and Virginia (Suomi et al. 2003). In a Western Kentucky hospital, operations were sometimes postponed when ladybeetles appeared in the sterile operating room. MALB may also cause inhalant allergic reactions in humans. These allergies clear up once the lady beetles are removed (Jones et al. 2002; Koch et al. 2006; Ray et al. 2004).

Apart from being a household nuisance, MALB can be a pest of fruit production, especially as a contaminant in wine production (Pickering et al. 2004; Galvan et al. 2006). The MALB taint is reported in northeastern United States and southern Canada (Pickering et al. 2004; 2005). When crushed or disturbed, adult MALB release a yellow fluid called hemolymph. If included in wine or juice this fluid can affect the odour, taste and flavour of wine. This contamination may result in the complete loss of the wine and/or increased production costs from additional time and labour needed to control MALB (Galvan et al. 2008; Galvan et al. 2007, Pickering et al. 2006a). In 2001, MALB were identified as being harmful to the Ontario grape and wine industry. The MALB problem occurs mainly with its presence on fruit clusters or containers upon delivery and inclusion in the subsequent processing sequence (Ker et al. 2006).

MALB are mainly observed in the vineyards 2-3 weeks before harvest and are attracted to the ripening grapes as a late source of sugar. MALB search for sugar-containing food sources prior to moving to aggregation sites and this may be the main reason for being attracted to damaged wine grapes, as adult MALB are not able to directly break the grape berry skins (Galvan et al. 2008). The grapes are usually injured by wasps, birds and splitting. Splitting is caused by a sudden increase in absorption and/or adsorption of water, atmospheric humidity or temperature. Depending on the number or clusters damaged, MALB can easily infest up to 65% of all clusters for some varieties (Galvan et al. 2006b; 2008). When the beetles are crushed along with the grapes, the hemolymph released causes off-aromas and off-flavours and can affect the wine or juice characteristics. The MALB problem mainly occurs in late ripening varieties such as Cabernet Franc, Cabernet Sauvignon, Riesling, Vidal and Vignoles .These cultivars are prone to cracking due to their thin skins can also be heavily infested (Anonymous, 2005). MALB affect the aroma of Concord grape (*Vitis labruscana* Bailey) juice at levels as small as 0.5MALB/kg (Ross et al. 2007).

Methoxypyrazine

When agitated or disturbed, MALB engage in “reflex bleeding” during which a yellow fluid is released. The fluid contains alkaloids used for defense and 3-alkyl-2-methoxypyrazines used as an aggregation pheromone (Galvan et al. 2008). Methoxypyrazines belong to the pyrazine family, a class of nitrogen-containing heterocyclic compounds are important flavour ingredients (Buchbauer 2000). As aroma compounds, pyrazines are identified in such heated foods as bread (Mulders et al. 1973), different meats (Wassermann 1972), baked potatoes (Buttery et al. 1973) and coffee (Bondarovich et al. 1967). These pyrazines are formed during the Maillard reaction from the reaction between reducing sugars and amino acids They are also present in a range of nuts and fresh vegetables including green bell pepper, beans, and asparagus where they contribute to green, earthy, herbaceous, vegetative and bell pepper attributes (Boubee et al. 2000; Pickering et al. 2007). The human olfactory threshold for methoxypyrazine is two parts per trillion in water (Cai et al. 2007).

Gas chromatography/mass spectrometry (GC/MS) is a common technique used to identify pyrazines. However, the detection of volatiles at low concentrations for complex matrices first requires an extraction and preconcentration step in sample preparation procedures. Extraction by cation-exchange resin, liquid-liquid extraction, C-18 SPE extraction and solvent extraction are some of the techniques used to concentrate methoxypyrazines from various juices. Solid-phase microextraction (SPME) is also used because it is a reusable sample preparation technique suited for rapid quantitative and qualitative analyses (Cai et al. 2007). Along with GC, mass spectrometry also offers good sensitivity for the detection of compounds in trace amounts (Cudjoe et al. 2005).

Cudjoe et al. (2005) used headspace gas chromatography-mass spectrometry to identify and determine methoxypyrazines in ladybeetles. MALB were found to produce 2-isoproryl-3-methoxypyrazine (IPMP) in a slightly greater concentration while 2-isobutyl-3-methoxypyrazine (IBMP) and 2-sec-butyl-3-methoxypyrazine (SBMP) were found in equal amounts (Figures1to 3). Cai et al. (2007) used headspace sampling along with SPME combined with multidimensional GC-MS-O and identified thirty-eight compounds released from live MALB, specifically four odour active compounds, including IPMP, SBMP, IBMP and 2,5-dimethyl-3-methoxypyrazine (DMMP).

The presence of methoxypyrazine in MALB infested wine did led to a clear conclusion as which methoxypyrazine is the main compound contributing to undesirable characteristics. Pickering et al. (2005) examined the influence of MALB on red and white wine composition and aging. Although IBMP and IPMP were identified in the tainted wine, the presence of IPMP was concentration dependent and increased as the number of beetles in red and white wine juices increased. The IPMP concentration was higher in white wines as opposed to red wines, but after 11 mo of aging, the concentration of IPMP decreased in all treatments. Galvan et al. (2008) also found SBMP, IPMP and IBMP in MALB infested wine when using headspace sampling coupled with SPME.

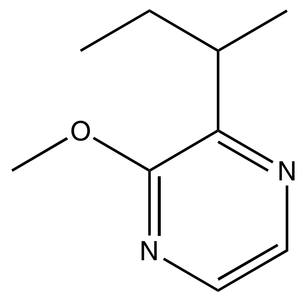

Figure 1: 2-sec-butyl-3-methoxypyraine (SBMP)

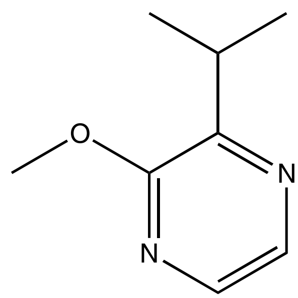

Figure 2: 2-isopropyl-3-methoxypyrazine (IPMP)

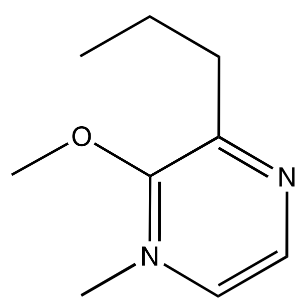

Figure 3: 2-isobutyl-3-methoxypyrazine (IBMP)

(NIST Chemistry 2005)

While Pickering et al. (2005) identified IPMP as the key compound for the off-flavour in wine, Galvan et al. (2008) found IPMP, SBMP or IBMP were not related to the to the MALB off-flavour. These authors suggested that in MALB infested wine, alkaloids or terpenes may be responsible for the off-flavours and off-aromas. However, in both of these studies, different types of wines were investigated along with different vinification techniques, tasting panels and extraction methods. These differences may be responsible for the different results. Pickering et al. (2005) used White Bourgeon and Red Bergamais grapes while Galvan et al. (2008) investigated Frontenac and Leon Millet grapes. From Pickering et al. (2005) and Galvan et al. (2008) methoxypyrazines may manifest differently in different grape cultivars.

Methoxypyrazines occur in some *Vitis vinifera* grapes and their wines as part of varietal character mainly the vegetative aroma (Allen et al. 1995). Allen et al. (1999) stated isobutyl methoxypyrazine is the main contributor to the vegetative aroma. However, isopropyl methoxypyrazine can still have sensory importance in red wines. Isobutyl-2-methoxypyrazine (IBMP), the compound causing the bell pepper characteristic, is found in wines Sauvignon Blanc, Cabernet Franc and Cabernet Sauvignon wines (Belancic et al. 2007). Along with IBMP, 3-isopropyl-2-methoxypyrazine (IPMP) has also been identified in some grape cultivars.

Methoxypyrazine concentration decreases during ripening, reaching a basal level before harvest. A high methoxypyrazine concentration in grapes at harvest is associated with immaturity and can impact the final wine quality (Belancic et al. 2007). The concentration of methoxypyrazine depends on climate, sun exposure and vine vegetative growth and yield (Allen 2001; Belancic et al. 2007). IBMP is sensitive to light and temperature (Prouteau et al. 2004); therefore, as the berries are exposed to more light, the final pyrazines levels decrease. In warm areas, the concentration of IBMP in grapes can decrease while increasing in cooler climates. In Cabernet Sauvignon grapes, IBMP is located mainly in the stems, skin and seeds. During ripening, IBMP decreases in the stems and seeds and increases in the skins (Boubee et al. 2002).

Substituted pyrazines have a broad range of aroma properties including green, earthy, nutty, roasted bell pepper woody. Pyrazines not only contribute to aroma but to flavour as well such as bell pepper and potato (Maga et al. 1973). The flavour perception is a consequence of the interaction between the flavour molecule and a specific receptor localized in the olfactory mucosa. Pittet et al. (1974) suggested an unsaturated nitrogen containing heterocycle as a general structure for pyrazines with preferably an isobutyl group at position 2 and a methoxy group at position 1 to give the bell-pepper odour. Any other substitutions do not favour the bell-pepper odour. Parliment et al. (1973) suggested the size of the alkyl group, mainly 6-9 carbon atoms also confers the bell-pepper aroma. 2-methoxy-3-alkylpyrazines have generated a great deal of interest amongst researchers mainly due to the powerful aroma and taste characteristics of these substituted pyrazines (Cudjoe et al. 2005). Buttery et al. (1970) described the green bell pepper odour as characteristic of 2-isobutyl-3-methoxypyrazine (IBMP). However, 2-alkyl-3-methoxypyrazines are also associated with the vegetal and herbaceous flavour found in Cabernet Sauvignon, Merlot, and Sauvignon Blanc wines.

Sensory Evaluation

Sensory evaluation of methoxypyrazines is challenging due to small concentrations found in grapes and wines (Allen et al. 1991). The human olfactory threshold for methoxypyrazine in water is two parts per trillion (Cai et al. 2007). However, despite the low threshold, the presence of MALB in juice and wine impacts the resulting sensory properties. Pickering et al. (2007) evaluated the detection threshold of 2-isopropyl-3-methoxypyrazine in two white wines, Chardonnay and Gewürztraminer, and a red wine blend of Baco Noir and Marchel Foch wines. Detection threshold is the lowest concentration at which a stimulus can be detected. IPMP was added to the wines and the thresholds were determined using the ascending forced choice method. Orthonasally, Chardonnay had the lowest threshold of 0.32 parts per trillion, next was the red wine blend with a threshold of 1.03 parts per trillion and then the Gewürztraminer with 1.56 parts per trillion. Retronasally, Gewürztraminer had a lowest threshold at 1.15 parts per trillion compared to the red wine blend with a threshold of 2.29 parts per trillion.

The threshold for IPMP ranged from 0.32 parts per trillion to 2.29 parts per trillion, with the difference in threshold attributed to sensitivity differences between individuals. Galvan et al. (2007) examined the sensory threshold of MALB in Frontenac wine grapes. One treatment included MALB added at 3.0 MALB/L to containers with grapes. The containers were agitated for 45 sec to mimic disturbance expected during grape harvest. MALB were removed before the wine was processed. Another treatment included MALB added at selected concentrations to grapes with wine made from MALB-contaminated grapes. The MALB were removed during racking. No significant differences were observed between the control wine (0 MALB/L) and the wine agitated to mimic reflex bleeding from 3 MALB/L. Galvan et al. (2007) concluded reflex bleeding from 3.0 MALB/kg grapes was not enough to taint the wine. However, in the MALB suggesting when MALB are incorporated into the winemaking process, the resulting wine is perceived as tainted.

MALB may be incorporated into grapes during juice processing. Pickering et al. (2008) examined the detection threshold for IPMP in Concord and Niagara grape juice. A standard stock solution of (1mg/ml concentration) of IPMP was prepared and concentrations ranging from 0.16 parts per trillion to 97.7 parts per trillion were added to both Concord and Niagara grape juice. Orthonasally and retronasally, Niagara grape juice had a lower threshold of 0.74 parts per trillion and 0.84 parts per trillion, respectively compared to Concord grape juice threshold of 1.11 parts per trillion and 0.84 parts per trillion, respectively. The presence of methyl anthranilate in the Concord grape juice may be a possible reason as to why a higher detection threshold was observed. These results supported the earlier results of Pickering et al. (2007b). Ross et al. (2007) investigated the orthonasal aroma threshold for MALB in Concord grape juice. MALB were incorporated into the juice making process. MALB concentrations increased from 0.5 MALB/kg to 4.5 MALB/kg, the number of correct responses increased. The threshold was 1.8 MALB/kg supported the Pickering et al. (2004) study where the presence of MALB in red and white wines were perceived as the concentration increased from 1 MALB/kg to 10 MALB/kg of grapes.

The influence of MALB on sensory properties of white and red wine was studied by Pickering et al. (2004). MALB wine concentrations were 0, 1 and 10 MALB/L. During racking, MALB were removed from the wines. Panelists evaluated the wine for aroma, taste and flavour. The wines were assessed 10 weeks after bottling. In white wine, at 1 MALB/L, bell pepper aroma and peanut flavours increased in intensity compared to the control wine. No significant differences were noted with the other attributes. At 10 MALB/L, bell pepper, asparagus and peanut aromas and flavours increased. In red wines, at 1 MALB/L, the bitter intensity increased when compared to the control wine, with no significant differences noted for any of the other attributes. At 10 MALB/L, plum and cherry aroma and sweet flavour intensities decreased while peanut, asparagus/bell pepper and earthy/herbaceous aromas and flavours as well as acid/sour and bitter intensities increased. Red berry, plum and cherry aroma and red berry flavour intensities were significantly lower in 10 MALB/L wine compared to the 1 MALB/L wine. All three tastes, sweet, acid/sour and bitter were affected by the presence of MALB in red wines while none were affected in white wines. The MALB attributes were more intense orthonasally than retronasally. With retronasal evaluation, the temperature and the intake of air may have affected the intensity of the attributes.

Pickering et al. (2007) determined which processing stages were critical to the development of MALB characteristics in wine. Stages of the winemaking process were investigated including agitation, crush/destem, whole bunch press and direct addition to juice. During agitation, MALB were added in closed pails along with grapes and rolled for 45 sec and removed. The grapes were then processed by microvinification techniques. During crush/destem , MALB were added in closed pails along with grapes and rolled for 45 sec, but were not removed after agitation in the pails. For whole bunch press, without crushing/destemming, MALB were added with the grapes and processed by microvinification techniques. Finally, during direct addition to juice, MALB were added to the pressed juice after it had been cold settled. The results of the difference test and orthonasal detection threshold test showed that control wine could be identified from crush/destem whole bunch press and direct addition to wine. Through sensory testing, the threshold for the white wine was 1.53 MALB/kg of Riesling grapes, while that for red wine was 1.8 MALB/kg of Red Bergamais grapes. However, this information cannot be generalized for all red and white grape varieties.

Differences in Sensitivity

For each of the senses, human sensitivity varies. Physiologically, age influences sensitivity (Amerine et al. 1979). Humans are more sensitive to sweet, bitter and salty taste between the ages of 20 and 40 than 40 and 60.However, age has little effect on sourness (Amerine et al. 1979). Along with age, experience, culture and variability among individuals are factors which may influence sensitivities. Flavour release depends on the influence of volatilization of aroma compounds. The volatilization of the compounds can be influenced by formulation and processing and also during product storage and consumption. Thermodynamics and kinetics also control the flavour release from food (Voilley, 2006).

Unlike solid foods which may be in the mouth for 30 to 60 sec, liquids are swallowed quickly and the release of aroma takes place during a short interval. Therefore individual variation in swallowing, jaw and tongue movements and saliva flow may have an effect on aroma and flavour releases (Boelrijk et al. 2006). The overall flavour perception varies depending on taste responses and perception. One main taste which is thought to have an impact on overall flavour perception is bitterness (Prescott 2006). Bitterness sensitivity exhibits the most variation among humans compared to the other tastes. Hedonic assessments are used to determine sensitivity to phenylthiocarbamide (PTC) and 6-n-propythiouracil (PROP), organic compounds which can taste very bitter to tasteless depending on individual's genetics (Prescott 2006). Sensitivity to PROP is genetically inherited and allows classification of individuals into three groups, nontasters (NT), medium-tasters (MT), and super-tasters (ST) (Pickering et al. 2004; Bartoshuk, 2000). Super-tasters find the PROP compound very bitter. The sensitivity to bitterness may act as an indication of sensitivity to other tastes. Supertasters gave higher ratings than non-tasters and medium tasters to the saltiness and bitterness in salty/bitter mixtures, the bitterness in the sweet/bitter mixtures and sweetness in the sweet/sour mixtures. Thus this may affect perception of attributes in the grape juice. However, culture and associative learning may also affect preferences (Prescott 2006).

Pickering et al. (2004) investigated the mouthfeel sensation of red wine and its association with sensitivity to PROP. Acidity, bitterness and astringency were rated lower by NTs than by MTs or by STs. In the case of yogurt, cream cheese and orange juice, no differences were observed with the group in sweetness but super tasters were able to discriminate smaller variations in lower taste concentrations than NT (Prescott et al. 2004).

Management of MALB

MALB can aggregate in households and inside buildings. Compounds such as camphor and menthol repel MALB on the outside of buildings but the effects lasts only 48 hours (Riddick et al. 2000; Galvan et al. 2006a). Riddick et al. (2004) showed MALB were repelled by N,N-diethyl-3-methylbenzamide (DEET), a widely used insect repellent. A formulation of DEET plus paraffin had repellent activity for 23 days in the laboratory, however the effectiveness needs to be tested in the field (Kenis et al. 2008). Recommendations to prevent the entrance of MALB in buildings include checking all possible entry sites, sealing windows, cracks and small holes throughout the building with weather stripping, placing insect screening over windows, attic and exhaust vents. To remove large aggregates of MALB in buildings, the use of a broom and dust pan or a vacuum cleaner is recommended (Jones et al. 2002; Baniecki et al. 2004; Potter et al. 2005; Kenis et al. 2008).

Black light traps and other light traps can be used to capture MALB in dark places. However, these devices are not effective in controlling MALB in vineyards or orchards (Kenis et al. 2008). While MALB will not damage grapes, MALB are attracted to damaged grapes which may be caused by splitting. Growers may reduce splitting by irrigating for longer periods, selecting cultivars with a resistance or tolerance to splitting, or decrease the damage when pruning or spraying (Galvan et al. 2006). Managing MALB will be useful to the wine and juice industry.

Harvesting methods may reduce the number of MALB present on the grapes thereby decreasing the incidence of tainted wine or juice (Kenis et al. 2008, Anonymous 2005). Hand harvesting may a better option than mechanical harvesting as aggregations of MALB in grape clusters may be monitored during harvesting and infested grapes can be discarded. MALB can be removed by shaker clusters, by hand, or by using shaker tables and by floating clusters in water or vacuum clusters. These techniques can increase time, labour and cost of harvest (Galvan et al 2006c). Managing the surrounding crops and vegetation could also be critical because MALB found in vineyards and orchards in autumn likely come from nearby aphid infestations. In North America, damage in vineyards is often associated with the presence of soybeans infested by the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), one of the preferred preys of

MALB. The exact relationship between the proximity of soybeans, soybean aphids and MALB populations is not yet rigorously monitored (Kenis et al.2008).

Galvan et al. (2006a) tested the effect of insecticides to MALB under laboratory and field conditions. Based on field and laboratory studies, the insecticides carbaryl, bifenthrin, zetacypermethrin, thiamethoxam and imidacloprid showed either a toxic or repellent effect on MALB. However, only carbaryl and imidacloprid are labeled for use on wine grapes within seven days of harvest, the period during which MALB reach high densities. Although bifenthrin decreased the infestations of MALB, it can only be used on grapes 30 days before harvest. Most MALB populations significantly increase two weeks prior to harvest (Galvan et al. 2006a).

Spinosad and indoxacarb has shown to decrease the population growth of MALB (Galvan et al. 2005). MALB were treated with these insecticides at increased doses ranging from 10-25% of the field rate. Spinosad did not affect MALB survival but reduced MALB fertility. Spinosad causes involuntary muscle contractions, tremors and eventually paralysis of treated insects. Indoxacarb caused a decrease in the survival rate. Indoxacarb causes insects to stop feeding and go into mild convulsions or permanent paralysis (Tillman et al. 2001; Wing et al. 2000; Galvan et al. 2005). Although insecticides were successful in decreasing MALB, most insecticides have a pre-harvest interval of several weeks in order to be effective (Galvan et al. 2006). Therefore pre-harvest intervals may be a limiting factor when using insecticides.

Environmentally-friendly repellents push MALB away from structures or grape clusters during the fall and attractants, such as pheromones to attract MALB into outdoor traps have been studied (Riddick et al. 2000; Riddick et al. 2004; Brown et al. 2006).

Pheromones have been used for monitoring pest populations, trapping pest populations (lure and kill), confusing pests by saturating a crop environment with synthetic pheromones to prevent males locating females and manipulating or encouraging natural enemies (enis et al. 2008). Pheromones constitute only one category in the general term "semiochemicals", which includes both inter- and intraspecific signals (Silverston 1981). Pettersson et al. (2005) showed MALB from Coleoptera: Coccinellidae use an autumn aggregation pheromone to locate overwintering sites. However, more research is needed on the chemical nature of the aggregation pheromones. Brown et al. (2006) detected volatiles emitted from live beetles using whole-air sampling and solid phase microextraction (SPME) and identified the compounds using GC/MS. (-)-β-caryophyllene was found only in females.

Verheggen et al. (2007) showed (-)-β-caryophyllene and (E)-β-farnesence could be used as biological controls for MALB. A bioassay technique was used which employed a ventilated plastic box male and female MALB aggregated to the side with (-)-β-caryophyllenene and (E)-β-farnesence. In an olfactometer assay, male and female MALB were attracted to (E)-β-farnesence, while only males were attracted to (-)-β-caryophyllenene. The use of (-)-β-caryophyllenene and (E)-β-farnesence to control MALB is a possibility but more research is still needed.

Remediation

Although control methods for MALB have been investigated, the incorporation of MALB in juice and wine may be unavoidable. Thus remediation techniques for dealing with MALB tainted juice and wines are important. The problems associated with MALB taint are new to the juice and wine industries. Research is being performed to identify a way in which juice or wine can be remediated.

Fining agents such as bentonite, gelatin, isinglass and activated charcoal are commonly used in the wine industry to adjust and stabilize wine quality, including the prevention and/or removal of visual, olfactory and gustatory faults (Pickering et al. 2005). Pickering et al. (2006) subjected 1 and 10 MALB/L infested red and white wines to six treatments. The treatments were bentonite, activated carbon, oak chips, deodourized oak chips and a UV light treatment (wavelength 254nm and 18.3 W). Results showed activated charcoal significantly reduced IPMP concentrations in the treated white wine when compared to the untreated wine. Based on sensory evaluation, using a trained panel, the activated charcoal was not found to reduce the MALB attributes. The presence of oak chips significantly decreased the undesirable asparagus flavour and also decreased the other aroma and flavour attributes such as peanut, earthy as well as bitter taste. In red wine, deodourized oak reduced the IPMP concentration in the untreated wine while the other treatments had no effect. Based on sensory evaluation, bentonite, charcoal, oak chips and deodourized oak all reduced the asparagus flavour in the red wine. Oak chips reduced MALB taint in both red and white wine. However, oak cannot be used in all wine styles. Aromatic and delicate varieties such as Muscat and Gewürztraminer are not normally oaked.

Government Policies

While management and remediation studies are still being conducted, the incidence of MALB contaminated wines and juices are still prevalent. The United States Department of Agriculture Inspection Procedures for foreign material in the fruit and vegetable division stated the limits established for foreign material are predicated by the nature of the material, the impact upon the consumer, the capability of the industry to remove such material under acceptable practices and the possible harmful effect of the material. The Food and Drug Administration Center for Food Safety and Applied Nutrition (FDA/CFSAN) set defect action levels for certain products which pose no inherent hazard to health. These limits have been established because it is economically impractical to grow, harvest or process raw products that are totally free of non-hazardous, naturally occurring, unavoidable defects. However, the action levels are not intended to cover poor manufacturing practices. Products containing foreign material pose no inherent hazard to health are classified as classes 1, 2 and 3 while products found to contain harmful material are classified as class 4.

Class 1 foreign material is classified as not readily discernible either visually or organoleptically and generally requires microscopic examination for detection. Examples include but are not limited to fly eggs, maggots, insects and insect fragments 2 mm or less in length, mold, rodent type hairs and feather barbules. Class 2 foreign material is classified as generally discernable without microscopic examination but requires careful organoleptic examination of the product for detection. Examples include but are not limited to maggots, larvae and insects less than 7 mm in length but more than 2 mm. Class 3 foreign material is classified as readily discernible and/or highly objectionable from an aesthetic standpoint. Examples include but are not limited to worms and large insects 7 mm and larger in length, strands of hair, paper, excessive or coarse sand that seriously affects the eating quality. Class 4 foreign material is classified as readily discernible, highly objectionable and potentially harmful. Examples include but are not limited to machine grease, oil, stones, nails, glass, sharp metal, silvers, sharp wood splinters, thorns, burrs (sharp/hard), puncture vine, barley barbs and loose solder.

Products containing off-flavours should be certified as sub-standard which is defined as the product contains a definite off-flavour but is deemed fit for consumption. Examples of these off-flavours include fermented (winey), or musty. Another classification is graded not certified (GNC), which is considered to be “out of condition” or otherwise deemed unfit for consumption. Examples of these classifications are putrid or flat sour. According to the FDA Food Defect Action Level handbook, berries can have a defect of mold, insects and larvae. The action level for mold occurs when the average mold count is 60% or more while for insects and larvae the action level occurs with an average of 4 or more larvae per 500 grams of berries or an average of 10 or more whole insects or equivalent per 500 grams. These are significant to the sensory appeal of the fruit.

However, these limits are not tested but based upon how they may impact the fruit and what may be deemed acceptable to the consumer. The effects of MALB have been researched in wine and in Concord grape juice. However, more research on MALB and its detection limits and how changes the sensory properties of beverages may give a better indication of the complexity of the problem.

CHAPTER THREE

MATERIAL AND METHODS

Concord and Niagara grapes and Multicoloured Asian Lady Beetles

In September 2006, live Multicoloured Asian Lady Beetles (MALB) identified by an "M" inscribed just preceding the wing covers (elytra) were collected from cornfields in Yakima Valley, WA. The beetles were immediately placed in cold storage (4º C) until needed for the Niagara and Concord grape juice production. In September 2006, at commercial maturity, Concord grapes were harvested from the Irrigated Agriculture Research and Extension Center (IAREC), Washington State University Prosser, Washington. In September 2007, Niagara grapes were harvested from Grandview, WA.

Preparation of Grape Juice

Concord grape juice

The basic grape juice flow diagram is shown in Figure 4. Concord grape juice was prepared at Irrigated Agriculture Research and Extension Center, (Prosser, WA) in triplicate. Concord grape juice was prepared without (control) and with (treated) the addition of MALB. For each batch, 34 kg of grapes were weighed and crushed with a small rotary stemmer crusher. Crushed grapes and pressing paper (Weyerhaeuser, WA) were scooped alternately into a jacketed kettle. For the treated batches, the kettle was layered with grapes, pressing paper and MALB.

For Concord grape juice, eight hundred MALB each added to replicate 1 and replicate 2 and four hundred were added to replicate 3. Following the addition of MALB, the grape mash was heated to 60ºC in a steam jacketed kettle. Pectinase enzyme

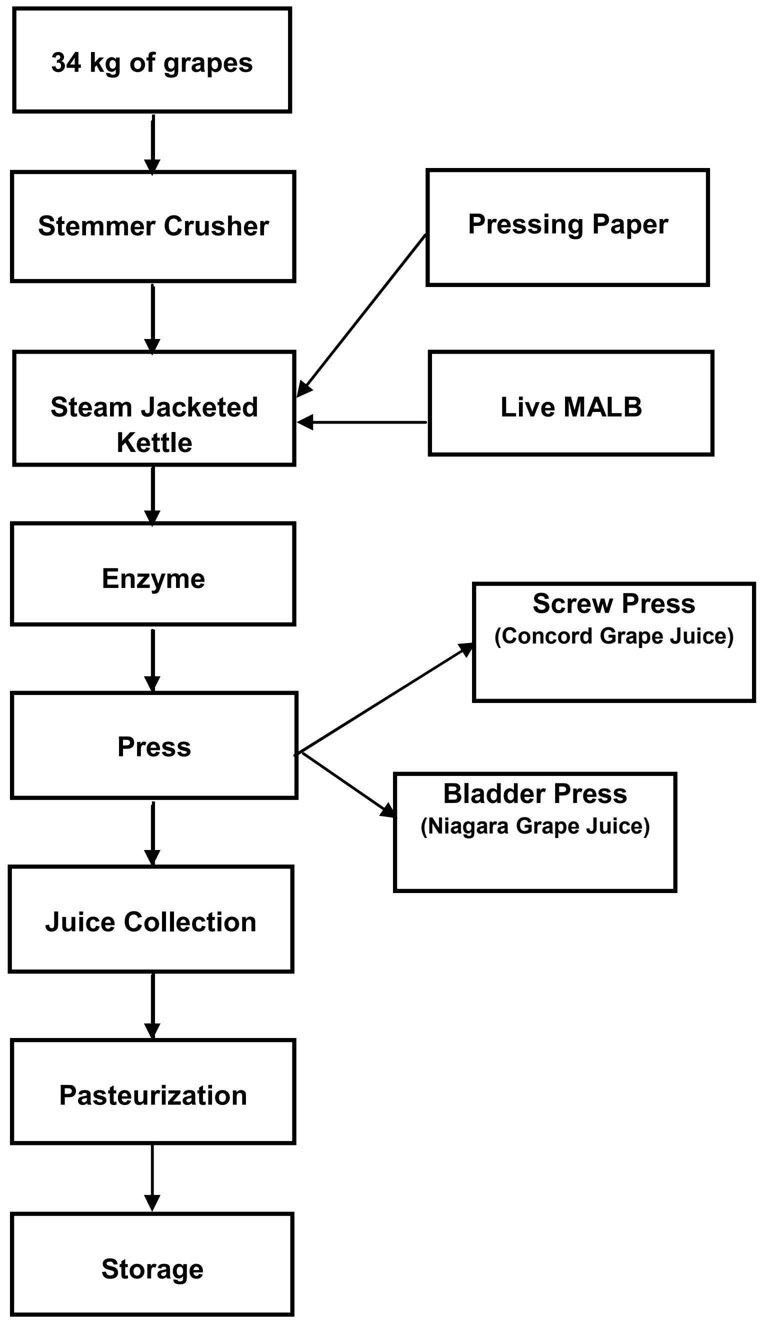

Figure 4. Basic Flow Diagram of Grape Juice Making Process for Concord and Niagara grape juice

Scottzyme Pec 5 L (Scott Laboratories, Petaluma, CA) was added at a concentration of 0.37 ml/75 lbs of Concord grapes. The slurry was mixed at intervals over 35 min and maintained at a constant temperature of 60 ºC to allow enzyme activity. The grape slurry was transferred into a screw press, the juice was collected and pasteurized for 2 min at 185 ºF. The juice was stored at (4° C) overnight. The following day, the juice was distributed among 1-L glass Ball (Muncie, IN) canning jars and boiled in a water bath at 100 ºC for 6 min.

The final MALB concentrations for each replicate of Concord grape juice was 34.5 MALB/L (replicate 1), 44.7 MALB/L (replicate 2) and 18.09 MALB/L (replicate 3). The total yield of Concord grape juice was 110L from 34 kg of grapes. Concord grape juice was stored at -35°C.

Niagara grape juice

The basic grape juice flow diagram is shown in Figure 4. Niagara grape juice was prepared at Washington State University Pilot Plant (Pullman, WA) in duplicate due to limited number of beetles. Niagara grape juice was prepared without (control) and with (treated) the addition of MALB. For each batch, 34 kg of grapes were weighed and crushed with a small rotary stemmer crusher. Crushed grapes and pressing paper (Weyerhaeuser,WA) were scooped alternately into a jacketed kettle. For the treated batches, the kettle was layered with grapes, pressing paper and MALB.

For Niagara grape juice, 212 MALB were added to replicate 1 and replicate 2. Following the addition of MALB, the grape mash was heated to 60 ºC in a steam jacketed kettle. Pectinase enzyme Scottzyme Pec 5 L (Scott Laboratories, Petaluma, CA) at a concentration of 0.37 ml/34 kg of Niagara grapes, was added and mixed at intervals over

35 minat a constant temperature of 60ºC to allow enzyme activity. The grape slurry was transferred to a bladder press run at 45 psi. The juice was collected, distributed among the glass canning jars and pasteurized at 100°C for 15 min. The final concentrations were 8.76 MALB/L (replicate 1) and 9.15 MALB/L (replicate 2). The total yield of Niagara grape juice was 87 L from 34 kg of grapes. Niagara grape juice was stored at -35°C.

Analytical Determinations

Analytical determinations of pH, TA and Brix were conducted according to AOAC methods (2000). The pH was measured using an Accumet AB15 Plus pH meter (Fisher Scientific, USA). For titratable acidity (TA), 0.1 N sodium hydroxide solution was prepared by boiling 1L of deionized water in an Erlenmeyer flask. After the water cooled, 4g of NaOH pellets (J.T Baker, Phillipsburg, NJ) were added and allowed to equilibrate to room temperature overnight. The sodium hydroxide solution was attached to a Titro-Line Easy autotitrator (Schott Instruments, Deutschland, Germany).The grape juice solution was titrated to a pH end point of 8.4. Brix was determined using a Pocket Refractometer (ATAGO, Japan). Data analysis was conducted using one-way ANOVA (XLSTAT, Addinsoft, Paris, France) with mean separation using Tukey's HSD.

Sensory Studies

Aroma Detection Threshold of Concord grape juice

Panelists were recruited from Washington State University, Pullman, WA through advertisements posted in the Food Science and Human Nutrition Building and online campus announcements. To limit any bias, limited information about the study was provided. Panelists were awarded a small non-monetary incentive for participation. Panels were conducted in individual sensory booths in the sensory facility in the Food

Science and Human Nutrition Building at Washington State University (Pullman, WA). Twenty-four panelists, between the ages of 18-65 participated in each of the sensory panels. The panel was repeated on a second day. Before each panel, all panelists signed an Informed Consent Form which indicated the study was approved by the Washington State University Institutional Review Board.

The first panel consisted of 22 females and 2 males, while the second panel consisted of 14 females and 10 males. The detection threshold was determined using the American Society of Testing and Materials (ASTM) 3-Alternative Forced Choice (3-AFC) method. Using this method, a series of triangle tests were performed. Specifically, three juices were presented; two control juice samples and one MALB (Meilgaard et al. 2004). The individual selected which juice was different from the other two. For Concord grape juice, three scale steps were used with a three- fold dilution factor between steps. This scale was selected to limit panelist olfactory adaptation. The grape juice concentrations used (0.5, 1.5 and 4.5 MALB/L) were made by serial dilutions of MALB juice with control juice (no MALB). The final MALB concentrations used were based on a study conducted by Ross et al. (2007).

Panelists were first presented with water and instructed to sniff the water prior to sample evaluation. Panelists were then presented with three flights of three randomly coded juices per flight (nine juices total). Juices (15ml) were presented in 25 ml glass beakers (Pyrex, USA) and covered with a plastic Petri dish. The lowest concentration (0.5 MALB/L) was presented first followed by 1.5 MALB/L and 4.5 MALB/L (final concentration). Panelists were instructed to firmly place their index finger on the Petri dish and gently swirl the beaker several times. The panelists were then instructed to remove the lid and take 2-3 short sharp sniffs. Panelists were instructed to indicate which of the grape juice samples were different based only on aroma. Due to the nature of the forced choice method, the panelists were required to guess if they could not discriminate. Panelists were given a forced break of fifteen sec between each flight. Red lights were used to mask any colour differences.

Concord Grape Juice after 11 Mo of Storage

The 3-AFC evaluation of grape juice aroma panel was replicated on the same Concord juice following 11 mo of storage at -35°C. Thirty panelists (10 males and 20 females) were recruited as described previously. The procedure and concentrations used were as described previously.

Niagara Grape Juice

For Niagara juice, recruitment was similar to that of the Concord grape juice panel. Panelists were presented with a non-monetary incentive. Twenty-four panelists, between the ages of 18-65 participated in each of the two sensory panels. The first panel consisted of 11 males and 13 females, the second panel consisted of 10 males and 14 females. Before each evaluation, panelists signed an Informed Consent Form which indicated the study was approved by the Washington State University Institutional Review Board. As with Concord grape juice, the 3-AFC method was used.

The Niagara juice was prepared using a 3-fold dilution factor between MALB concentrations. Welch's 100% Niagara grape juice (Concord, MA) was mixed in a 1:1 ratio with control grape juice and used for dilution. Preliminary trials were used to select the appropriate scale range and concentrations for the threshold tests. The first trial used concentrations of 0.17, 0.5, 1.5 and 4.5 MALB/L presented in 25 ml glass beakers

(Pyrex) and presented under white lights. Results indicated more than fifty percent of the panelists correctly identified all the MALB concentrations. It was thought this result was either due to too high concentrations or colour differences between the MALB/L juices and the control juice (no MALB).

Due to the dilution the MALB juice was visually different from the other juices and this may have resulted in selection by the panelists. A second preliminary trial was conducted to determine appropriate MALB concentrations could be used and whether panelists were able to distinguish differences based on characteristics of the juice or colour differences. MALB juice concentrations of 0.06 and 0.17 MALB/L were presented in Libbey cobalt blue wine goblets (Toledo, OH). Panelists indicated no apparent colour differences between the juices. Based on these preliminary trials, a four scale step with a 3.6 fold dilution step between concentrations was selected. The final concentrations selected for the aroma threshold study were 0.06, 0.21, 0.78, 2.80 MALB/L.

Panelists were first presented with water and instructed to sniff prior to juice evaluation. Panelists were presented with four flights of three randomly coded juices (12 juices total). Juices (15 ml) were presented in blue SOLO plastic cups (Highland Park, IL) covered with large Petri dishes. The lowest concentration (0.06 MALB/L) was presented first followed by 0.21, 0.78 and 2.80 MALB/L (highest concentration). Panelists were instructed to firmly place their index finger on the Petri dish and gently swirl the beaker a few times. Instructions followed that the lid was to be removed and 2-3 short, sharp sniffs taken. Panelists indicated which of the three juices was different. Due to the forced choice method, the panelists were required to guess if they could not discriminate. Panelists were given a forced break fifteen sec between each flight. Yellow lights were used to mask any colour differences.

Statistical Analysis

Data were collected using a computerized sensory software (Compusense five, Release 4.6, Guelph, Ontario, Canada) and analyzed using the BET method (ASTM E 679 -04 (2004). The individual BET was calculated by taking the geometric mean of the largest concentration correctly identified by the panelist and the next concentration not correctly identified. If a panelist missed all the concentrations, the best estimate threshold was calculated using the highest concentration missed and the next hypothetical concentration. For Concord grape juice, the next largest hypothetical concentration after the highest concentration was 13.5 MALB/L, while for Niagara it was 10.0 MALB/L.

If a panelist correctly identified all three concentrations, the BET was calculated by taking the lowest concentration correctly identified and the next lower hypothetical concentration. For Concord grape juice, the next lowest hypothetical concentration was 0.17 MALB/L while for Niagara it was 0.02 MALB/L. The group BET was calculated as the geometric mean of the individual BET values. Differences in individual BET between the two replicate panels for Concord and Niagara grape juice were analyzed using a Student's t-test in Excel. Significance was defined at $p \leq 0.05$.

Trained Panel

Concord grape juice

Panelists were recruited from WSU, Pullman, WA through advertisements posted in the Food Science and Human Nutrition building and online campus announcements. Panelists (9 females, ages 22-55) were selected based on their interest and availability in the study. Panelists consumed fruit juice at least three times a week. All of the training and evaluation sessions were conducted in the sensory facility of the Food Science and Human Nutrition Building at WSU, Pullman, WA. At the end of each training session, a small non-monetary gift was distributed while a WSU gift was awarded at the end of the study.

The panelists attended five one-hour training sessions over a period of two weeks. Panelists were given limited information about the study so as to limit bias. During the first session, panelists signed an Informed Consent Form which showed the study was approved by the WSU Institutional Review Board. The initial attributes used in training were generated based on a small focus group of experienced sensory staff and students. During training, panelists were familiarized with the techniques used for evaluations (Table 1) and attributes used to describe the juice (Table 2). Panelists were informed about the importance of cleansing their palates and were instructed to sniff water before evaluating the juices for aroma and use the water and crackers before and after in-mouth evaluations. Panelists were cautioned to take breaks between juices to avoid fatigue of the senses. Panelists were introduced to the 15-cm unstructured line scale with low, medium and high anchors of each standard. Panelists were instructed how to use the scale effectively to rate the intensities of the juice flavours.

Standards and preparation of standards are shown in Table 2. Panelists evaluated standards for the following attributes. The medium and low standards were made by 2-3 fold dilutions of the original standard. During session 1, panelists evaluated two (15 ml) randomly coded juices containing 0 MALB/L and 34.54 MALB/L presented in 25 ml glass beakers (Pyrex, USA). The intensities of all the attributes were rated on a 15-cm

Table 1. Techniques used by trained and untrained panelists to evaluate aroma, taste, mouthfeel and flavour of Concord and Niagara grape juice (n = 9, n = 8).

SENSORY ATTRIBUTE	TECHNIQUE
Aroma	
Detected by the olfactory system	Gently swirl the container. Take 2-3 short, sharp sniffs
Taste	
Stimulated by nerve endings on tongue	Sip juice, swish in mouth 1-2 sec. Swallow or spit juice into cuspidor
Mouthfeel	
Coating of the mouth	Sip juice, hold in mouth for 7-10 sec coating the entire mouth, spit juice into cuspidor
Flavour	
	Gently slurp the juice, swish juice gently around the mouth , swallow a little

Table 2. Final list of standards, standard preparations and descriptions for Concord grape juice as evaluated by the trained panel (n=9).

ATTRIBUTE	REFERENCE COMPOSITION[a]	DESCRIPTION
Aroma and Flavour		
Earthy/Musty/Dirty	50 g dried dirt presented in a plastic container	Aroma and flavour of dirt or mulch
Plum	4 tsp plum jam in 100 ml juice	Aroma and flavour of Plum preservatives
Grape	100ml of Concord grape juice	Aroma and flavour of Concord grape juice
Vegetal	20 mm of fresh bell pepper, flame heated for 20 sec, soaked in grape juice for 20 min + 30 ml canned asparagus juice	Aroma and flavour of freshly cut (green) bell pepper and canned asparagus juice
Taste		
Sweet	12.5 g sucrose/L	Intense sensation on the tip of the tongue
Sour/Acid	3.3 g tartaric/L	Intense sensation at the sides of the tongue
Mouthfeel		
Astringent	1.2 g tannic acid and 0.5 g alum dissolved in 1L milli-Q water	Drying or puckering feeling of the palate
Chalky	6.67 g crushed antacid tablets	Feeling of fine particles felt inside the mouth

[a]Standards prepared in 100 ml of Welch's 100% Concord grape juice unless otherwise stated. Standards represent "high" anchor

line scale. Evaluations were discussed by the group and terms were added, dropped and/or combined.During session two, the peanut standard was removed and grape standard was introduced. Bell pepper and asparagus standards were combined to form a vegetal descriptor. These suggestions were based on the panelists' comments. The modified earthy standard was prepared in 100 ml of Welch's 100% Concord grape juice (Concord, MA). All of the standards and techniques were reviewed at the beginning of the session. The panelists evaluated three (15 ml) randomly coded juices (0, 5.8, and 7.2 MALB/L) for aroma, taste, mouthfeel and flavour. Evaluations of the juices were discussed by the group and feedback was given to the group.

During session three, all the standards and techniques were reviewed and three 15 ml juices (0, 2.0 and 5.0 MALB/L) were evaluated for aroma, taste, mouthfeel and flavour. Evaluations were discussed by the group. At the end of session 3, the mean intensities and standard deviations were analyzed for the panel for each attribute using XLSTAT (AddinSoft, Paris, France). Attributes with a standard deviation of 3 or above indicated which attributes needed more practice. For each attribute, outlying panelists were identified by three or more units above or below the mean score for the attribute. Hence, this indicated the panelists and attributes which needed more training.

During session four, panelists received feedback about their performances individually and in relation to the group. Panelists were encouraged to discuss which attributes provided difficulty and tips from panel leader and other group members were given. Panelists were encouraged to revisit standards. Panelists evaluated four 15 ml juices (0, 1.0, 2.0 and 4.0 MALB/L) for aroma, taste, mouthfeel and flavour. For session five, panelists only revisited standards with which they were not comfortable. This was based on the feedback session at the end of each training session. If panelists were either marking the attribute too high or too low on the scale based on the mean value, they were encouraged to practice some more with the standards. Panelists evaluated six 15 ml samples (0, 0.45, 0.9, 1.8, 3.6, MALB/L) presented in a random order for aroma, taste, mouthfeel and flavour.

After the training sessions, panelists participated in two formal evaluation sessions in individual testing booths using red lights used to mask any potential colour differences. Prior to evaluation, standards were provided for the panelists who needed to refresh. Grape juice concentrations (0, 0.45, 0.9, 1.8, 3.6, 7.2 MALB/L) were prepared two hours prior to evaluation. Six (15 ml) individual juice samples were presented to the panelists in a random serving order and evaluated using a computerized 15-cm line scale presented on the sensory evaluation software (Compusense five, Release 4.6, Ontario, Canada). Panelists had a forced break of 120 sec between juices three and four and a break of 15 sec between the other juices. Data were collected by Compusense five Release 4.6 (Guelph, Ontario, Canada) and analyzed by two-way analysis of variance (ANOVA) with replicate, panelist and MALB concentration as main effects. The interaction of replicate x panelist, replicate x MALB concentration, and panelist x MALB concentration were determined for the sensory attributes. Tukey's HSD for mean separation using the program STATA (College Station, TX) was used to determine significant differences. Significance was defined at $p \leq 0.05$.

Niagara grape juice

Panelists were recruited from WSU (Pullman, WA) through advertisements posted in the Food Science and Human Nutrition building and online campus announcements. Panelists (1 male, 7 females, ages 23-64) were selected based on their interest and availability in the study. Panelists consumed fruit juice at least three times a week. All the training and evaluation sessions were conducted in the Sensory Facility of the Food Science and Human Nutrition building at Washington State University (Pullman, WA). At the end of each training session, a small non-monetary incentive was given while a WSU gift was awarded at the end of the study. To limit any bias, limited information about the study was provided.

Panelists attended nine one-hour training sessions. In session one, panelists were given a three digit-coded sample (15 ml) of Niagara grape juice (9.15 MALB/L) and asked to describe its attributes. A list of previously generated attributes (Table 3) was distributed and the panelists added attributes to the list. Panelists agreed on four aroma and flavour attributes (earthy/musty/dirty, grapey, honey and vegetal), two tastes (sweet and sour) and one mouthfeel (astringent). Panelists were informed about the importance of the use of palate cleansers (water and crackers) and taking breaks between juice evaluations. Panelists were introduced to the 15-cm unstructured line scale and instructed how to effectively use it to rate the intensities of the juices. Panelists were instructed on the technique used to evaluate aroma (Table 1). Standards and standard preparations are shown in Table 3. Vegetal, earthy and grapey standards were evaluated by the panelists. First, the high intensity standards were prepared followed by the medium and low intensity standards using a 3 or 4 dilution of the high standard. The dilutions were used to mimic the intensities in the Niagara grape juice. Honey was not evaluated in the initial session as panelists decided to add it to the list of attributes in session 2.

Table 3. Final list of standards, standard preparations and descriptions for Niagara grape juice (n = 9).

ATTRIBUTE	REFERENCE COMPOSITION[a]	DESCRIPTION
Aroma		
Earthy/Musty/Dirty	5 g of diced mushrooms in 100 ml of water	Aroma and flavour of dirt or mulch
Honey	22 g of clover honey in 100 ml juice	Aroma and flavour of honey
Grape	100 ml of Niagara grape juice	Aroma and flavour of grape juice
Vegetal	10 ml of asparagus juice + 60mm square of fresh bell pepper, flame heated for 20 s and soaked in grape juice for 20 min	Aroma and flavour of asparagus and bell pepper
Taste		
Sweet	11g sucrose in 100 ml of juice	Intense sensation on the tip of the tongue
Sour/Acid	2.11g tartaric acid in 100 ml of juice	Intense sensation at the sides of the tongue
Mouthfeel		
Astringent	0.7 g of tannic acid + 0.2 g of alum in 100ml juice	Drying or puckering feeling of the palate

[a]Standards in 100ml of Welch's 100% Niagara grape juice unless otherwise stated. Standards represent "high" anchor

Four randomly coded three-digit juice samples (15ml) of concentrations 9.15, 4.5, 1.5 and 0.5 MALB/L were evaluated for the aroma attributes. Due to limited supply of control juice, these concentrations were diluted with a 1:1 ratio of control (0 MALB/L) and Welch's 100% Niagara juice concentrate (Concord, MA). Evaluations were discussed by the group and feedback was given to panelists about each of the juice samples evaluated. In session two, panelists agreed on the list of attributes which adequately described the juice. The aroma technique and standards were revisited. Taste and mouthfeel techniques (Table 1) and standards (sweet, sour and astringent) at high, medium and low anchors were introduced and evaluated using the 15 cm unstructured line scale. Panelists evaluated five (15 ml) juice samples at concentrations, 8.76, 4.0, 1.33, 0.44, 0.15 MALB/L for aroma, taste and mouthfeel. All evaluations were discussed as a group. In session three, the group revisited the aroma, taste and mouthfeel techniques along with the standards. Honey, earthy/musty/dirty, grapey and vegetal standards at high, medium and low anchors were evaluated with the flavour technique. Three (15ml) juices at concentrations (0.5, 1.5, 4.5 MALB/L) were evaluated for aroma, taste, mouthfeel and flavour. All evaluations were discussed afterwards as a group.

In session four and five, panelists revisited all of the standards and techniques used to evaluate the juice. Panelists discussed which attributes were difficult to grasp. The panel leader and other panelists provided personal cues which allowed them to better identify the attributes. In session four, five juices (15ml) of concentrations 0.09, 0.29, 0.91, 2.82, 8.76 MALB/L were evaluated. Six (15ml) juices (0, 0.5, 1.80, 3.6, 4.5, 7.2 MALB/L) presented in session five were evaluated for the attributes. All evaluations were discussed afterwards as a group. In session six, panelists were introduced to individual testing booths with a computerized version of the 15-cm unstructured line scale, presented on computerized sensory software (Compusense five, Release 4.6, Ontario, Canada). The panelists were allowed to familiarize themselves with the standards prior to entering the individual testing booths. However, it was advised to limit the number of standard as this would fatigue the senses while in the booths. In the testing booths, the panelists evaluated six (15ml) Niagara grape juices of concentrations 0, 0.45, 0.9, 1.8, 3.6, 7.2 MALB/L. Water, crackers and a cuspidor were provided in the booths. Panelists had a forced break of 90 sec between juices three and four and a break of 15 sec between all the other juices.

Data were collected and analyzed for mean intensities and standard deviations by Compusense five Release 4.6 (Guelph, Ontario, Canada). Individual feedback about performance was e-mailed to panelists. For each attribute, a standard deviation of 3 or greater indicated the group needed further training in that attribute. In addition, each panelist was assessed individually for each attribute and panelists outside of the mean group were informed of whether they were correctly rating the intensity of the attribute.

In session seven, panelists focused on the attributes which needed more training as outlined in the individual feedback. Standards were provided for those who needed to refresh. Panelists evaluated six (15ml) juices at concentrations (8.76, 4.0, 1.33, 0.44, 0.15 MALB/L). All evaluations were discussed as a group afterwards. In session eight, the panelists evaluated six juice (9.15, 3.51, 1.35, 0.52, 0.20 MALB/L) in the individual booths. Panelists relied on training, therefore no standards were provided in the training room. Data were collected and two-way ANOVA was performed using panelist and MALB concentrations as the main effect. The interaction between panelist x MALB concentration was determined. Tukey's HSD was calculated to determine if there were significant differences among MALB concentrations and the attributes. The standard deviations of the attributes were analyzed. The marks of each panelist were located and the outliers were identified. Feedback was e-mailed to each panelist, using panelists numbers for anonymity. Analyses were performed on Compusense five Release 4.6 (Guelph, Ontario, Canada).

In session nine, panelists discussed concerns about the panel, suggesting an increase in juice volume and an increase in forced breaks. Panelists briefly reviewed techniques and evaluated six juices (9.15, 3.51, 1.35, 0.52, 0.20 MALB/L). The results were discussed at the end of the session. Panelists participated in two replicate evaluation sessions in individual testing booths. Control grape juice (0 MALB/L) was used to dilute the MALB juice. Panelists were presented with six (25 ml) randomly coded three digit juices of concentrations, 0, 0.12, 0.32, 0.97, 2.92, 8.76 MALB/L. A break of 150 sec was given between juices three and four, and 40 sec was given between all the other juices.

Data were collected by Compusense five Release 4.6 (Guelph, Ontario, Canada) and analyzed by two-way ANOVA with replicate, panelist and MALB concentration as main effects. The interaction of replicate x panelist, replicate x MALB concentration, panelist x MALB concentrations were determined for all the sensory attributes. Tukey's HSD for mean separation using the program STATA (College Station, TX) was used to determine significant differences. Significance was defined at $p \leq 0.05$.

Consumer Rejection Panel

The consumer rejection panel for Concord grape juice was conducted following 12 mo in storage at -35 °C. Niagara grape juice consumer rejection panel was conducted three mo after juice preparation and storage at 3°C. For both panels, sixty panelists, ages 18-61 were recruited. For Concord juice, the panel was composed of 39 females and 21 males, while for Niagara juice, the panel was composed of 23 males and 31 females. Panelists were recruited from WSU and surrounding communities through advertisements posted in the Food Science and Human Nutrition Building and online campus announcements. Panelists were presented with small incentives after completing the panel. Prior to evaluations, panelists cleansed their palates with water and crackers. Panelists were instructed to use the aroma evaluation technique described on the computer screen.

When conducting in-mouth evaluations, panelists were informed about the option to swallow or expectorate. Water was provided to sniff between each juice to refresh the olfactory senses. Panelists were instructed to use the water and crackers between juices for the evaluation of taste. Five randomly coded paired juices (15ml each), consisting of one control (0 MALB/L) and one MALB sample were presented in 25 ml glass beakers (Pyrex, USA) starting at the lowest concentration and increasing until the highest concentration. The concentrations used for Concord and Niagara juices were 0, 0.45, 0.9, 1.8 and 3.6 MALB/L. Each paired juice was presented one at a time. Panelists were instructed to first evaluate the aroma and then proceed to an in-mouth evaluation. After the evaluation of each paired juice, panelists indicated which juice was preferred. All the ballots were presented by sensory evaluation software (Compusense five Release 4.6, Guelph, Ontario, Canada). Control juice (No MALB) was used for dilution. Red lights were used to mask any potential colour differences. Data were collected from Compusense five Release 4.6 (Guelph, Ontario, Canada). Results for significant rejection were based on the binomial distribution for paired comparison (Roessler et al. 1978). Significance was defined as $p \leq 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

Analytical Results of Concord and Niagara Grape Juice

The analytical values (pH, titratable acidity and Brix) for Concord and Niagara grape juice are shown in Table 4. The pH values of Concord grape juice ranged between 3.12 and 3.15. In Concord grape juice, at both 0 MALB/L and 4.5 MALB/L, pH was 3.12. The pH values for MALB Concord grape juice concentrations 0, 0.5 and 1.5 MALB/L were significantly different ($p \leq 0.05$) from each other. For Niagara grape juice, as the MALB concentration increased from 0 MALB/L to 4.5 MALB/L, the pH values decreased from 3.32 to 3.17 ($p \leq 0.05$).

As the concentration of MALB in Concord and Niagara grape juice increased, the Brix values decreased. For Concord grape juice, at 0 MALB/L, the Brix value was 18.8, while at 4.5 MALB/L, the Brix value was 18.5 ($p \leq 0.05$). For Niagara grape juice, at 0 MALB/L, the Brix value was 17.0, while at 4.5 MALB/L, the Brix value was 16.2. The percent titratable acidity (% TA) for Concord grape juice also decreased as the MALB concentration increased. At 0 MALB/L, the TA was 1.04, while at 4.5 MALB/L the TA was 0.89 ($p \leq 0.05$). For Niagara grape juice, as the MALB concentration increased, the TA increased. At 0 MALB/L the TA was 1.20, while at 4.5 MALB/L, the TA was 1.28 ($p \leq 0.05$).

According to the Food Drug and Administration Center for Food Safety and Applied Nutrition, the approximate pH of Concord grape juice is between 2.80 and 3.00, while it is between 2.80 and 3.27 for Niagara grape juice. In agreement with this study, when examining the impact of MALB on the pH of red and white wine, Pickering

Table 4. Values pH, TA and Brix of Concord and Niagara grape juice at concentrations of 0, 0.5, 1.5 and 4.5 MALB/L. Mean values are presented with standard deviation. Different letters indicate significant differences ($p \leq 0.05$) as determined by Tukey's HSD.

MALB conc (MALB/L)	Concord	Niagara
pH		
0	3.12 ± 0.01^a	3.32 ± 0.01^a
0.5	3.15 ± 0.01^c	3.22 ± 0.01^b
1.5	3.14 ± 0.01^b	3.20 ± 0.01^c
4.5	3.12 ± 0.00^a	3.17 ± 0.01^d
Brix		
0	18.8 ± 0.00^a	17.0 ± 0
0.5	18.6 ± 0.06^c	16.9 ± 0
1.5	18.7 ± 0.00^b	16.7 ± 0
4.5	18.5 ± 0.00^c	16.2 ± 0
% TA		
0	1.04 ± 0.02^a	1.20 ± 0.01^b
0.5	0.92 ± 0.00^b	1.26 ± 0.01^a
1.5	0.94 ± 0.06^b	1.28 ± 0.02^a
4.5	0.89 ± 0.06^c	1.28 ± 0.02^a

et al. (2004, 2005) did not observe a change in pH due to the presence of MALB. In this study, the presence of MALB affected the analytical values of both Concord and Niagara grape juice. A possible reason for the changes in the pH, TA and Brix may be due to the fluid secreted by MALB. The presence of alkaloids and methoxypyrazines in the MALB hemolymph, in addition to carbohydrates and protein in the MALB themselves may have an effect on the resulting pH, TA and Brix values of the juice. However, more research is needed on this area as it is still unclear as to the direct effect of the MALB and on the basic composition of the juice.

Aroma Threshold

Concord grape juice

The aroma threshold panels conducted in Fall 2006 are represented as session 1 and 2, while the panel conducted in Fall 2007 after 11 mo of storage is represented as Session 3 (Figure 5). The percentages of correct responses for all sessions, as well as the best estimate thresholds (BET) calculated using the method outlined by ASTM E 679-04 (2004) are shown in Figure 5. In session 1 and 2, as the MALB concentration increased from 0.5 MALB/L to 4.5 MALB/L, the percentage of correct responses increased. In Session 1, at 0.5 MALB/L, 29% of the panelists correctly identified the MALB sample, at 1.5 MALB/L, 46% of the panelists correctly identified the sample and at 4.5 MALB/L, 63% of the panelists correctly identified the sample. A similar trend was observed in session 2. At 0.5 MALB/L, 29% of the panelists identified the MALB sample, at 1.5 MALB/L, 50 % of the panelists correctly identified the sample and at 4.5 MALB/L, 58% of the panelists correctly identified the MALB tainted sample. In session 3, at 0.5 MALB/L, 41% of the panelists correctly identified the MALB sample,

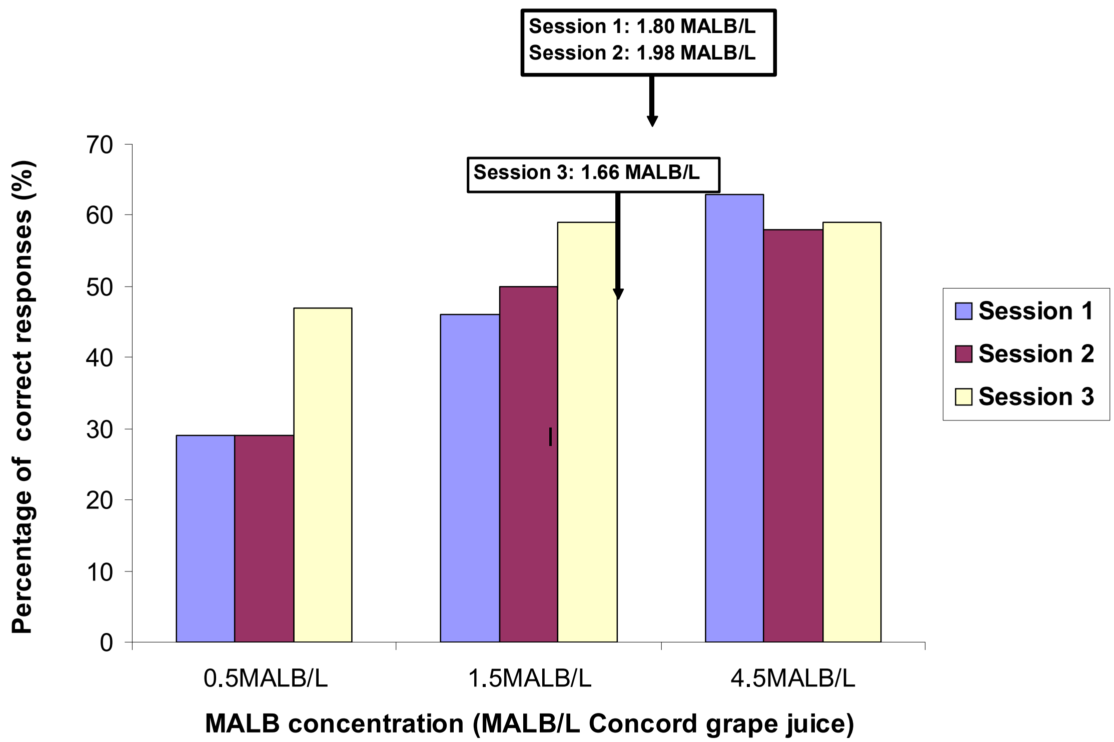

Figure 5. Percentage of correct panelists at concentration 0.5, 1.5 and 4.5 MALB/L Concord grape juice for aroma threshold. Replicate panels (session 1 and 2), were conducted in Fall 2006 (n = 24 panelists per session). Session 3 was conducted in Fall 2007 (n = 17 panelists). The group best estimate threshold (BET) for each session is presented above.

at 1.5 MALB/L, 59% of the panelists correctly identified the sample and at 4.5 MALB/L, 59% of the panelists correctly identified the sample.As the MALB concentration increased, the number of correct responses increased. This pattern observed in session 1 and 2 was similar to a study conducted by Ross et al. (2007). In that study, the 3-AFC method was used to identify a threshold for MALB-tainted Concord grape juice. The MALB concentrations used were 0.5, 1.5 and 4.5 MALB/kg grapes. At a concentration of 0.5 MALB/kg grapes, 39% of the panelists correctly identified the MALB juice, at 1.5 MALB/kg, 55% of the panelists correctly identified the MALB juice and at 4.5 MALB/kg grapes, 67 % of the panelists correctly identified the MALB juice.

Pickering et al. (2004) also observed that as the MALB concentration increased from 0 MALB/L to 10 MALB/L, aroma intensities increased. In red wines at 1 MALB/L, panelists only noticed bitterness as the only significant attribute. However at 10 MALB/L, peanut, asparagus/bell pepper and earthy/herbaceous aromas increased while fruity aromas such as plum and cherry decreased.BET values slightly differed between session 1 and session 2; however, these differences were not significant ($p \geq 0.05$). The group BET for session 1 was 1.80 MALB/L and 1.98 MALB/L in session 2 (Figure 5). Due to the possibility of having panelists from session 1 participate in session 2, it was expected the BET for session 2 to be lower than session 1. Lesschaeve et al. (1996) stated known odours are more likely to be recognized than unknown odours.

Therefore, repeat panelists would have been familiar with the process and the odour associated with MALB. In session 1 and 2, panelists described MALB juice as musty, vegetal, and herbaceous, while in session 3, panelists described the aroma of the Concord grape juice as earthy, moldy, dirty, musty, vegetal, and potato when compared to the control juice. In a study conducted by Ross et al. (2007), panelists described MALB contaminated Concord juice as musty and dirty.The results of Concord grape juice after 11 mo of storage is represented in Figure 5 as session 3. Although the BET value was different from session 1 and 2, the values were not significantly different from each other.

Pickering et al. (2005) added MALB beetles at concentrations of 1 and 10 MALB/L to red and white wines and evaluated the sensory profile after 10 mo of aging. After 10 months the aroma and flavours were similar to newly bottled wine. As MALB concentration increased, peanut, bell pepper and asparagus flavours and aromas increased in white wine while in red wine peanut and asparagus/bell pepper flavours and aromas increased as MALB concentration increased. For both red and white wines floral and fruity intensities decreased as MALB concentration increased.

When MALB is disturbed or crushed, a yellow odour-active hemolymph (blood) containing alkylmethoxypyrazines is released. Alkylmethoxypyrazines are known to contribute vegetative, herbaceous, green bell pepper and earthy attributes to wines such as Cabernet Sauvignon and Sauvignon Blanc (Galvan et al. 2007). In these grapes, high concentrations of methoxypyrazine are associated with immaturity. Concentrations also depend on climate, sun exposure and vine vegetative growth and yield (Belancic et al. 2007). The methoxypyrazines in growing grapes, such as Carmenere, are sensitive to light and temperature.Therefore the more exposure to light ,the lower the methoxypyrazine levels (Belancic et al. 2007).

Pickering et al. (2005) examined the concentration of 2-isopropyl-3-methoxypyrazine (IPMP) and 2-methoxy-3-isobutylpyrazine (IBMP) with gas chromatography and showed these compounds decreased with aging. Stern et al. (1973) found that even though compounds may be present in large abundance in particular mixtures, their contribution to the total aroma may not necessarily be directly proportional to their concentration. The results observed with Concord grape juice after 11 mo of storage could have been compared to Niagara grape juice but due to limited resources, the experiment was not be performed.

The presence of MALB presented similar characteristics in Cabernet Sauvignon and Sauvignon Blanc (Belancic et al. 2007). In the present study, the MALB tainted juice differed from the control Concord grape juice, suggesting the presence of MALB may be detected by aroma. The objective of this study was to determine the detection threshold. Detection threshold or absolute threshold is the lowest stimulus that can be detected (Meilgaard et al. 2007). Significant differences were not observed between repeat sessions 1 and 2. Significant differences were expected for the threshold test of the grape juice after 11 mo of storage. Methoxypyrazine is a volatile compound which can be detected at a low threshold. With storage, it was suspected the detection of methoxypyrazine would change. Although the work of Pickering et al. (2005) did not support the present study, it should be noted Pickering et al. (2005) study used wine and this study used grape juice. The presence of ethanol increases the solubility of methoxypyrazine thus decreasing the concentration of methoxypyrazine in the headspace (Pickering et al. 2005; Hartman 2002).

Niagara grape juice

The repeat panels conducted in Spring 2008 are represented as session 1 and 2 in Figure 6. In session 1, as the MALB concentration increased from 0.06 to 2.8 MALB/L, the percentage of correct responses increased. In session 1, at 0.06 MALB/L, 42% of the panelists correctly identified the MALB sample, at 0.21 MALB/L, 58% of the panelists correctly identified the MALB sample, at 0.78 MALB/L, 63% of the panelists correctly identified the MALB sample and at 2.8 MALB/L, 79% of the panelists identified the MALB juices. A similar trend was observed in session 2. At 0.06 MALB/L, 33% of the panelists correctly identified the MALB sample, at 0.21 MALB/L, 29% of the panelists correctly identified the MALB sample, at 0.78 MALB/L, 58% of the panelists correctly identified the MALB sample and at 2.8 MALB/L, 71% of the panelists identified the MALB juices. A similar trend was observed with the detection threshold of Concord grape juice in this study and Ross et al. (2007).

As observed with the Concord aroma detection study, as the MALB concentration increased in Niagara grape juice, the MALB aroma became more noticeable. The group best estimate thresholds (BET) for session 1 and 2 were calculated by the method outlined by ASTM E 679-04 (2004). The group BET for session 1 was 0.46 MALB/L and 0.83 MALB/L for session 2 (Figure 6). As the concentration of the MALB increased in Concord and Niagara grape juice juices, the MALB aroma became more noticeable. Although the group BET values differed between the two sessions, no significant differences were observed ($p \geq 0.05$).

The selection of concentrations used to evaluate the BET for Niagara grape juice was determined through preliminary trials. In the initial trials, the same concentrations used for Concord grape juice (0.5, 1.5 and 4.5 MALB/L) were initially used for Niagara grape juice. However, due to the less intense aroma of Niagara grape juice, the MALB concentrations were easier to distinguish in Niagara than in Concord grape juice. More than 50% of the panelists correctly identified the MALB tainted juices. This may have been due to the nature of the juice. Subsequent preliminary trials for Niagara grape juice led to the following concentrations, 0.06, 0.21, 0.78 and 2.8 MALB/L. The aroma of Concord grape juice was more distinct than that of Niagara grape juice. Subsequently, in this study four concentrations were used since Niagara aroma seemed not as complex as Concord grape juice and also it helped to better indicate whether people were actually detecting a difference or guessing, thus making the BET value more reliable. In Niagara grape juice, differences between session 1 and 2 existed especially between the concentrations of 0.06 MALB/L to 0.21 MALB/L. In a forced choice method, panelists are given three juices, two juices are the same and one is different. Panelists must choose a sample perceived to be different although none may be clearly distinguishable. Panelists will answer based on their perception even though it may not be the correct answer (Meilgaard et al. 2006). The percentage of correct responses varied between Niagara and Concord grape juice for sessions 1 and 2. Niagara grape juice had greater percentage of correct responses than for Concord grape juice. Therefore, the BET value for Niagara grape juice was lower than Concord grape juice. Volatile and nonvolatile compounds of Concord and Niagara grapes have undergone study by other researchers. The aroma compounds in grape juice include methyl anthranilate, methyl and ethyl 3-hydroxybutanoate, ethyl 2-butenoate and 2,5,-dimethyl-4-methoxy-3 (2H)-furanone (Pickering et al. 2008; Schreier at al. 1981). However, the more intense aroma associated with Concord grape juice is mainly due to methyl anthranilate. The presence of this compound may be responsible for the difference in detection threshold between Concord and Niagara grape juice.

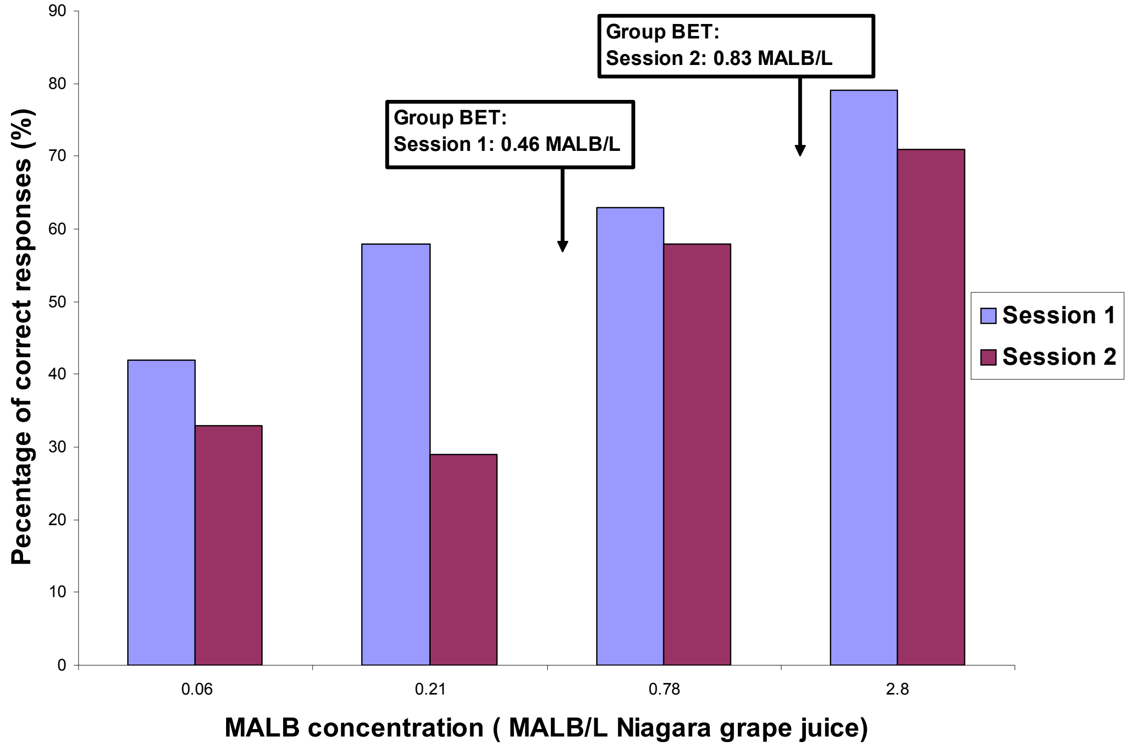

Figure 6. Percentage of correct panelists at concentration 0.06,0.21, 0.78 and 2.8 MALB/L Niagara grape juice aroma threshold. Replicate panels (session 1 and 2), were conducted in Spring 2008 (n = 24 panelists per session). The group best estimate threshold (BET) for each session is presented above.

The low odour threshold of Niagara grape juice compared to Concord grape juice was supported by Pickering et al. (2007), the orthonasal and retronasal threshold of 2-isopropyl-3-methoxypyrazine of white wine to be lower than in red wine. Pickering et al. (2008) also showed the orthonasal and retronasal threshold of Niagara grape juice was lower than in Concord grape juice. Based on the results of the present study, aroma is an effective method to identify the presence of MALB in grape juice.

Trained Panel

Concord grape juice

The sensory attribute F-values for sessions (replicate), panelist, MALB concentration and interactions between rep and panelists, rep and MALB concentration and panelists and MALB concentration for aroma and taste are shown in Table 5. Mouthfeel and flavour attributes are shown in Table 6. Significant differences ($p \leq 0.05$) were observed between the MALB concentrations for vegetal aroma and flavour. Tukey's HSD means separation (Table 7) shows which MALB concentrations were significantly different from each other. For vegetal aroma, panelists distinguished differences between concentrations 0 and 7.2 MALB/L, 0.45 and 7.2 MALB/L, 0.9 and 7.2 MALB/L and 1.8 MALB/L. For vegetal flavour, panelists distinguished differences between 0 and 7.2 MALB/L, 0.45 and 7.2 MALB/L, 0.9 and 7.2 MALB/L, 1.8 and 7.2 MALB/L, 3.6 and 7.2 MALB/L.

Tukey's HSD showed the intensity of the vegetal aroma of the MALB Concord grape juice increased from 0 MALB/L to 0.9 MALB/L. A decrease in intensity was observed at 1.8 MALB/L and the intensity increased at 3.6 MALB/L and 7.2 MALB/L. For vegetal flavour, a decrease in intensity was observed between 0 MALB/L and 0.45

MALB/L and an increase in the intensity was observed from 0.45 MALB/L to 7.2 MALB/L. Not all the attributes of the MALB juice increased with increasing MALB concentration and the attributes rated at the highest intensity varied with MALB concentration. At 0 MALB/L, the highest intensity attributes were earthy aroma, sweetness and sourness. At 0.45 MALB/L, sweetness and grape flavour were the highest intensity attributes, at 0.9 MALB/L and 1.8 MALB/L, sweetness and grape flavour were the highest intensity attributes. At 3.6 MALB/L sweetness was the highest intensity attribute and at 7.2 MALB/L, sweetness and sourness were the highest intensity attributes.

Results from this present study showed that although Concord grape juice had a strong and complex aroma and flavour, panelists distinguished differences in the juice at various MALB concentrations. Based on the aroma detection panel, it was expected as the MALB concentration increased, the intensity of all the attributes would increase. However, for vegetal aroma, a decrease in intensity was noticed between 0.9 MALB/L and 1.8 MALB/L. For vegetal flavour a decrease in intensity was noticed between 0 MALB/L and 0.45 MALB/L. The other attributes evaluated for MALB Concord grape juice were not significantly different from each other. Significant ($p \leq 0.05$) panelist effects were observed, suggesting a lack of consistency among the panelists. For this study, panelists were trained over nine 1-h sessions. While panelists should have been consistent with each other, trained panelists cannot be compared to expert panelists. A study conducted by Zamora et al. (2004) evaluated the performance comparison between trained assessors and wine experts using specific sensory attributes.

Table 5. Degrees of freedom and F-ratios from analysis of variance (ANOVA) of trained panel (n = 10) evaluation of Concord grape juice for aroma and taste attributes. Evaluations were made in replicate.

		Aroma			**Taste**		
Source of Variation	**df**	**Earthy**	**Vegetal**	**Grape**	**Plum**	**Sweet**	**Sour**
Rep	1	2.82	0.25	0.720	0.03	0.04	0.62
Panelist	9	1.34	6.37***	6.78***	12.27***	4.59***	5.12***
MALB/L	5	0.87	6.51***	2.81	1.55	0.38	1.30
Rep*Panelist	7	1.21	1.29	0.85	1.45	1.10	0.90
Rep*MALB/L	5	3.73**	1.20	2.870	0.83	1.65	0.21
Panelist*MALB/L	45	0.63	1.39	1.030	1.17	0.84	1.00

*, **, *** indicates significant p≤ 0.05, 0.01, 0.001 respectively

Table 6. Degrees of freedom and F-ratios from analysis of variance (ANOVA) of trained panel (n= 10) evaluation of Concord grape juice for aroma and taste attributes. Evaluations were made in replicate.

		Mouthfeel		**Flavour**			
Source of Variation	**df**	**Chalky**	**Astringent**	**Earthy**	**Vegetal**	**Grape**	**Plum**
Rep	1	0.76	6.98*	3.68	0.04	0.01	1.65
Panelist	9	11.18***	4.55***	4.70***	5.34***	23.42***	29.04***
MALB/L	5	0.86	1.49	1.43	8.10***	3.07	5.13
Rep*Panelist	7	3.17*	1.08	2.73	0.63	3.21	3.59
Rep*MALB/L	5	2.45	0.20	2.08	0.14	1.54**	0.40
Panelist*MALB/L	45	0.81	0.66	1.48	1.21	1.65	2.38

* ,**,*** indicates significant $p \leq 0.05, 0.01, 0.001$ respectively

Table 7. Mean values of aroma, taste, mouthfeel and flavour attributes of Concord grape juice evaluated by trained panelists (n=10). Evaluations were made in replicate. Different letter in the same row indicate significant differences among Concord grape juice analyzed by Tukey's HSD on a 15-cm unstructured line scale ($p \leq 0.05$).

		MALB concentrations (MALB/L)					
	Attributes	**0**	**0.45**	**0.9**	**1.8**	**3.6**	**7.2**
Aroma	**Earthy**	6.86[a]	5.22[a]	4.71[a]	5.48[a]	4.08[a]	5.42[a]
	Vegetal	2.69[a]	2.76[a]	3.57[a]	3.17[a]	5.11[ab]	7.17[b]
	Grape	4.55[a]	6.43[a]	5.99[a]	6.36[a]	6.14[a]	4.17[a]
	Plum	3.23[a]	3.19[a]	2.73[a]	3.89[a]	2.74[a]	1.97[a]
Taste	**Sweet**	7.37[a]	8.68[a]	8.65[a]	8.03[a]	7.63[a]	8.16[a]
	Sour	6.52[a]	5.28[a]	5.67[a]	5.70[a]	6.42[a]	7.46[a]
Mouthfeel	**Chalky**	1.12[a]	1.36[a]	1.53[a]	1.88[a]	1.09[a]	0.98[a]
	Astringent	4.78[a]	4.17[a]	3.89[a]	4.55[a]	6.47[a]	5.28[a]
Flavour	**Earthy**	4.68[a]	3.22[a]	3.61[a]	3.88[a]	4.28[a]	5.67[a]
	Vegetal	2.06[a]	1.67[a]	2.73[a]	2.88[a]	3.96[ab]	6.42[b]
	Grape	7.71[a]	7.81[a]	7.88[a]	7.56[a]	6.49[a]	6.07[a]
	Plum	3.42[a]	3.47[a]	2.36[a]	3.26[a]	2.91[a]	2.15[a]

Twenty-one volunteer panelists were trained for twelve hours over eight sessions on sensory attributes of Chardonnay wines. After training, experts demonstrated superior ability in discrimination among the wine juices. Wolters et al. (1994) studied the effect of training procedure on the performance of descriptive panels and observed that longer training periods produced product discrimination and agreement among panelists. Therefore, the panelist effect observed with this study may have been decreased by a longer training period. Along with more training, these attributes used in the trained panel could have been defined more and/or the panelists should have been allowed to generate attributes which may have helped them to better identify the off-characteristics in the juice.

Attributes such as earthy, vegetal and plum are not commonly associated with grape juice. Vegetal is a broad term used to describe food and beverage attributes. Preston et al. (2008) showed expert panelists were asked to define vegetal the following descriptors were used, bell pepper, veggie, green bean, cooked spinach, dill, stemmy, herbal, cabbage, broccoli, cooked vegetable. Trained panelists characterized “fresh” vegetal aroma by bell pepper and “cooked” vegetal aroma by canned vegetable, green beans, asparagus, corn and potatoes. These classifications were similar between trained and expert panelists. Thus, the general term vegetal is complex (Preston et al. 2008). Instead of using vegetal as a descriptor, the term could have been further defined making that characteristic more precise for panelists.

Apart from the aroma and flavour attributes being different, taste attributes such as sweet and sour attributes were difficult for most of the panelists. McBride et al. (1987) examined the perception of sugar-acid mixtures in lemon drink. Sucrose was added at concentrations of 2, 4, 8 and 16 % w/v into freshly squeezed lemon juice. Each sucrose concentration exerted a simple subtractive effect on acid intensity. The presence of sucrose raised the threshold concentration of the acid. Perceived acidity is strongly suppressed by sweetness, but sweetness is only weakly suppressed by acidity. Judging the sweetness of a sucrose/NaCl mixture is more demanding than does judging the sweetness of unmixed sucrose. When a mixture is evaluated, one sensory quality has to be ignored while the other is judged (Schifferstein 1984). People differ in their ability to detect sweetness (Amerine et al. 1976). This may be due to cultural and social differences.

Mouthfeel is a category of sensations occurring in the oral cavity (Lawless et al. 1998). It refers to the sensations activated by free nerve ending of the trigeminal nerve. In wine, mouthfeel includes the perceptions of astringency, temperature and prickling (Jackson, 2000). The mouthfeel attributes of chalky and astringency were difficult for panelists to grasp. This may have been due to unfamiliarity of these terms to the general population. Astringency, a tactile sensation described as puckering, rough or dry mouthfeel, due to the increased friction between the tongue and the surfaces inside the mouth is a difficult attribute to evaluate (Lea et al. 1978; Noble, 1999).

Astringency is due to the presence of phenolic compounds extracted from the grape seeds and skins. White wines show lower astringency because they generally have lower concentrations of phenolic compounds (Jackson, 2000). Perceived astringency can also increase with multiple ingestions. It is believed the dry mouth feeling is due to the precipitated salivary protein and glycoproteins coating the teeth and oral cavity (Jackson 2000). This can result in a carry-over effect which may lead to a higher perception of astringency (Guinard et al. 1986; Colonna et al. 2004; Lesschaeve and Noble, 2005).

Therefore differences in Concord MALB juice attributes may have differed but panelists may not have been able to detect it due to the carry-over effect.

Feedback for trained panels is a very important factor. Although feedback was given after every training session, panelists should have been allowed to have practice sessions in the booths to assess their consistency as well as strengths and weaknesses of the attributes used to describe the MALB tainted Concord grape juice. This would have highlighted the panelists who needed more training. If there is significant number of panelists associated with interaction effects, and judges are not reproducible, further training is needed (Lawless et al.1998).

Results showed the presence of MALB in Concord grape juice produced differences in the intensities of the attributes evaluated. Only vegetal aroma and flavour were significantly different between MALB concentrations. In this study, vegetal was classified as bell pepper and other green vegetables. Bell pepper is one of the odourants associated with IPMP which is a compound released by MALB. Methoxypyrazines are grape derived flavour compounds that contribute a very characteristic vegetative, herbaceous, bell pepper or earthy aroma to wine of some grape varieties. Vegetal was also a significant attribute in a study of MALB-tainted wine in a study conducted by Pickering et al. (2004). Panelists described the 10 MALB/L red and white wines to have a higher intensity of bell pepper and asparagus aromas and flavours when compared to 1 MALB/L and 0 MALB/L.

Pickering et al. (2005) showed IPMP to be the main compound responsible for characteristics in MALB-tainted wine. However, odour and chemical separation of IPMP and other methoxypyrazines from insect volatiles may have been a challenge. The odour intensity of the IPMP was the highest among the methoxypyrazines identified. However, when IPMP coeluted with 2-ethyl-1-hexanol, it was characterized by odours of rose and green. This may have been confused with IPMP. Cai at al. (2007) found four pyrazines secreted by lady beetles. 2,5 dimethyl-3-methoxypyrazine (DMMP) characterized by a moldy, earthy odour, 2-isopropyl -3-methoxypyrazine (IPMP) characterized by a peanut and potato odour, 3-sec-butyl-2-methoxypyrazine (SBMP) characterized by nutty, peanut and potato odours and 2 –methoxy-3-isobutylpyrazine (IBMP) characterized by a peanut, potato odour. Panelists analyzed the headspace volatiles released from live *H. axyridis* through a sniff port and described the odours as moldy, earthy, green pepper, potato, peanut and nutty.

Pure DMMP is not commercially available, therefore its contribution to MALB taint needs to be confirmed. However IPMP, SBMP and IBMP are released from live beetles as well as dead beetles and their identification has been confirmed with their commercial standards. IPMP was identified as the main compound of MALB aroma. Although the odour profile of the juice strongly suggested the presence of IPMP, an alternate compound such as 2-ethyl -1-hexanol may also contribute to the aroma characteristics of the MALB tainted juice. In order to confirm this, quantification of the compound will require further analysis by gas chromatography-mass spectrometry (GC-MS).

Niagara Grape Juice

Two-way ANOVA sensory attribute F values for session (replicate), panelists, MALB concentration and interactions between replicate and panelist, replicate and MALB concentration and panelist and MALB concentration are found in Table 8 for aroma and taste and in Table 9 for mouthfeel and flavour. The presence of MALB in Niagara grape juice had a significant ($p \leq 0.05$) impact on all the attributes evaluated including honey, vegetal and earthy aroma and flavour, sweetness, sourness and astringency. Tukey's HSD means separation (Table 10) shows which MALB concentrations were significantly different.

There was a general decrease in honey aroma and flavour and sweetness, while a general increase was observed in vegetal and earthy aroma and flavour and astringency. The highest and lowest intensities of each attribute occurred at various concentrations. For honey and earthy aroma and flavour, the highest intensities occurred at 0.12 and 8.76 MALB/L, respectively. The highest intensity vegetal aroma highest intensity occurred at 2.92 MALB/L while the corresponding flavour highest intensity was at 8.76 MALB/L. The intensity of grape flavour was the highest intensity at 2.92 MALB/L. The highest sweetness intensity occurred at 0 MALB/L and the highest sourness highest intensity occurred at 8.76 MALB/L. The presence of MALB at various concentrations affected various attributes. For each attribute, as the MALB concentration increased, there was not a corresponding increase or decrease in the intensity ratings. For 0 and 0.12 MALB/L, sweetness and sourness attributes were rated the highest. For 0.32 MALB/L, 0.97 MALB/L and 2.92 MALB/L, sourness and astringency attributes were rated the highest. For 8.76 MALB/L earthy aroma, sourness and astringency were rated the highest.

Although training was over three weeks, panelist effects were observed, indicating inconsistencies among the panelists in rating the attributes Replication effects were observed indicating panelists rated the attributes differently between sessions

Table 8. Degrees of freedom and F-ratios from analysis of variance (ANOVA) of trained panel (n = 8) evaluation of Niagara grape juice for aroma and taste attributes. Evaluations were made in replicate.

		Aroma			Taste	
Source of Variation	**df**	**Honey**	**Vegetal**	**Earthy**	**Sweet**	**Sour**
Rep	1	7.50**	17.04***	5.44*	2.90	13.14***
Panelist	7	10.29***	11.07***	11.13***	26.98***	23.34***
MALB/L	5	9.41***	5.48***	20.34***	3.68**	4.78*
Rep*Panelist	7	2.61	1.46	3.54**	0.77	3.44*
Rep*MALB/L	5	1.82	1.37	0.190	1.11	0.39
Panelist*MALB/L	35	3.61***	1.63	4.22***	1.29	1.73

*, **, *** indicates significant $p \leq 0.05, 0.01, 0.001$ respectively

Table 9. Degrees of freedom and F-ratios from analysis of variance (ANOVA) of trained panel (n = 8) evaluation of Niagara grape juice for mouthfeel and flavour attributes. Evaluations were made in replicate

		Mouthfeel	Flavor			
Source of Variation	**df**	**Astringent**	**Honey**	**Grape**	**Vegetal**	**Earthy**
Rep	1	0.03	2.68	5.06	12.90**	28.14***
Panelist	7	3.76*	19.30***	10.83***	20.31***	19.84***
MALB/L	5	4.93*	12.15***	8.93***	5.16*	11.79***
Rep*Panelist	7	2.21	3.31*	1.62	3.06*	8.07***
Rep*MALB/L	5	0.41	0.62	2.22	1.51	2.30
Panelist*MALB/L	35	0.96	1.39	1.69	1.16	1.43

*, **, *** indicates significant $p \leq 0.05, 0.01, 0.001$ respectively

Table 10. Mean values of aroma, taste, mouthfeel and flavour attributes of Niagara grape juice evaluated by trained panelists (n=10). Evaluations were made in replicate. Different letter in the same row indicate significant differences among Niagara grape juice analyzed by Tukey's HSD on a 15-cm unstructured line scale ($p \leq 0.05$).

		MALB concentrations (MALB/L)					
	Attributes	**0**	**0.12**	**0.32**	**0.97**	**2.92**	**8.76**
Aroma	**Honey**	4.29^{ac}	5.61^{a}	3.33^{bc}	3.31^{bc}	3.32^{bc}	2.35^{b}
	Vegetal	4.08^{ac}	2.88^{a}	4.85^{bc}	3.84^{a}	5.97^{b}	4.28^{ac}
	Earthy	6.33^{a}	3.65^{b}	4.88^{ab}	5.37^{ab}	6.03^{a}	10.26^{c}
Taste	**Sweet**	8.14^{a}	7.97^{a}	6.19^{ab}	6.83^{ab}	6.86^{ab}	5.71^{b}
	Sour	8.19^{a}	7.44^{a}	8.06^{a}	8.69^{a}	8.41^{a}	9.64^{b}
Mouthfeel	**Astringent**	6.70^{ac}	5.13^{a}	7.49^{a}	7.93^{bc}	7.78^{bc}	8.71^{bc}
Flavour	**Honey**	5.6^{ae}	6.91^{a}	5.30^{ce}	5.18^{de}	5.81^{ae}	3.14^{b}
	Grape	4.78^{ac}	4.17^{a}	3.89^{bc}	4.55^{ac}	6.47^{ac}	5.28^{b}
	Vegetal	2.64^{ac}	2.19^{a}	3.41^{a}	3.07^{a}	3.61^{bc}	4.11^{b}
	Earthy	4.20^{ac}	2.94^{a}	5.19^{c}	4.12^{ac}	5.19^{cd}	7.37^{b}

although the same MALB concentrations were evaluated. This suggested that panelists may not have been completely comfortable using the scale. Panelist x MALB interaction effect was significant for honey and earthy aroma indicating panelists did not use the scale similarly evaluating those two attributes.

From this study, results showed that for each attribute, significant differences were observed over MALB concentration and as the concentration of MALB increased, the highest rated attributes varied. Concord and Niagara grape juice have distinct odours sometimes described as "foxy" or "Welch's grape juice" odour. The main distinguishing compound is methyl or ethyl anthranilate which has been suggested as an important component of the juice characteristic (Nelson et al.1977; Power 1921). This odour is sometimes unpleasant to consumers (Amerine et al. 1976).

Nelson et al. (1977) showed the methyl anthranilate concentration varied between wine produced from Concord grapes and Niagara grapes. Concord wine has a higher concentration of methyl anthranilate (1.7 part per million) compared to Niagara (0.6 parts per million). Although the presence of alcohol in wine may cause some of the compounds to be more soluble, the present study gives an indication of the difference in intensity of compounds in Niagara and Concord grape juice. Other compounds such as sugars and phenolic compounds produced during ripening may be responsible for aroma and flavour attributes in grape juice. The difference in concentration of these products may affect the aroma and flavours of Niagara grape juice versus Concord grape juice (Moigne et al. 2008). However the interactions of these compounds need to be further researched.

The less intense aroma of Niagara grape juice coupled with a longer training period may have been determining factors in the differences observed between MALB concentrations in the Concord trained panel and Niagara trained panel. More significant differences were observed in the Niagara grape juice compared to the Concord grape juice. This was expected based on the results from the aroma threshold study.

Consumer Rejection Threshold

Concord grape juice

The percentage of panelists preferring the aroma and taste/flavour of the control Concord grape juice (No MALB) over the MALB juice is shown in Figure 7 and Figure 8, respectively. In both figures, the 5% significance is indicated by the dotted line. The percentage of panelists preferring the aroma of control Concord grape juice (0 MALB/L) over the MALB juice is represented in Figure 7. At 0.45 MALB/L, 63% of the panelists preferred the control juice over the MALB juice. At 0.9 MALB/L, 52% of the panelists preferred the control juice over the MALB juice. At 1.8 MALB/L, 77% of the panelists preferred the control juice over the MALB juice. At 3.6 MALB/L, 77% of the panelists preferred the control juice over the MALB juice. Since sixty panelists evaluated the juices, thirty-seven panelists (62%) was the critical number needed for preference to be significant at $p \leq 0.05$ (Roessler et al. 1978). The consumers did not have a significant preference for the MALB juice aroma over the control Concord grape juice (0 MALB/L) at a concentration of 0.9 MALB/L. However, panelists significantly preferred the aroma of the control Concord grape juice when compared to concentrations of 0.45, 1.80, 3.60 MALB/L.With the exception of MALB juice at a concentration 0.9 MALB/L, as the MALB concentration increased, the percentage of panelists preferring the control Concord grape juice (0 MALB/L) aroma increased. Consumers significantly rejected the MALB tainted Concord grape juice aroma at 1.8 MALB/L and above. From the aroma

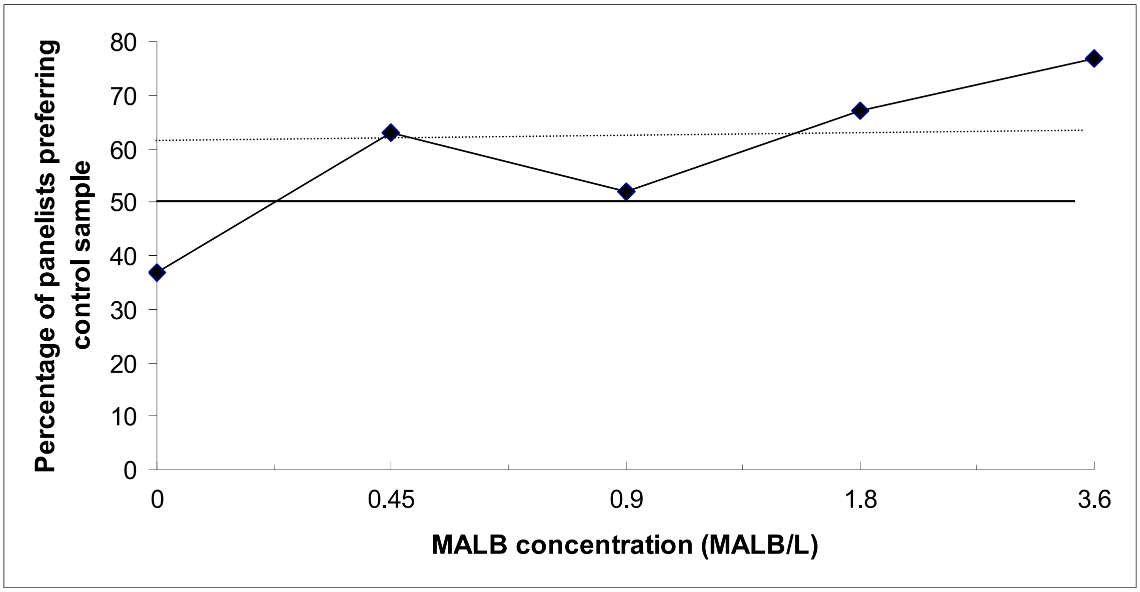

Figure 7. Percentage of panelists (n = 60) preferring the aroma of control Concord grape juice. The solid line (50%) represents no preference and the dotted line (62%) indicates significance at $p \leq 0.05$.

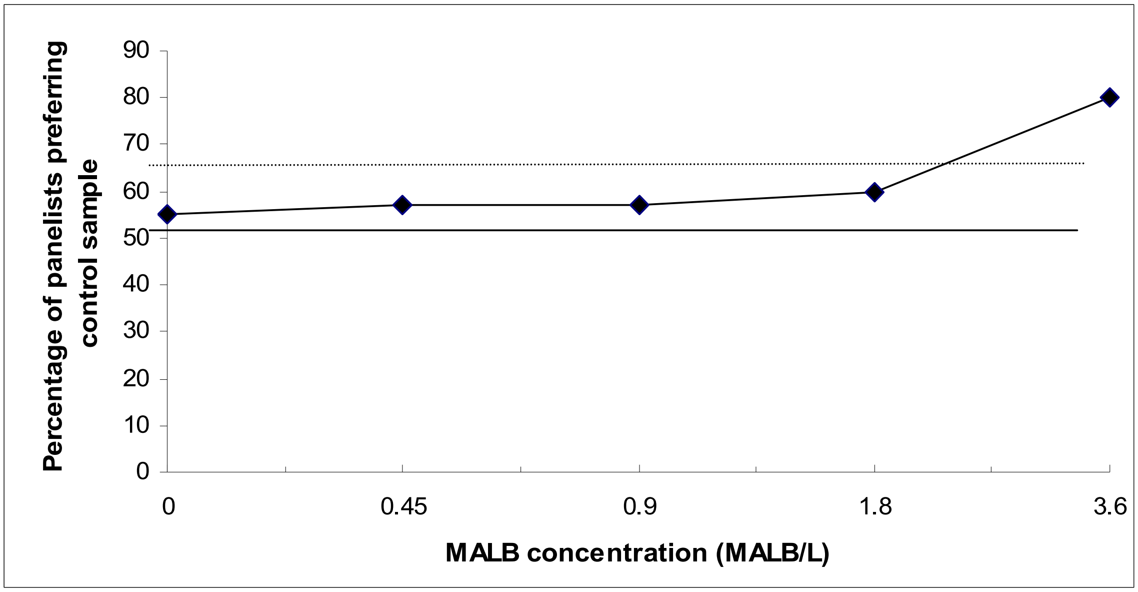

Figure 8. Percentage of panelists (n = 60) preferring the taste/flavour of control Concord grape juice. The solid line (50%) represents no preference and the dotted line (62%) indicates significance at $p \leq 0.05$.

threshold study the average threshold is 1.89 MALB/L, the concentration of significant rejection. Below the average detection threshold, consumers did not have a significant preference for the aroma of the control Concord grape juice versus the MALB tainted juice aroma. Therefore at concentrations below this threshold value some panelists could not detect a difference between MALB and control juice. Panelists described all of the MALB Concord grape juice as, having an off-aroma, pungent, musky, musty, dirty, earthy, dusty, vegetal and asparagus. Panelists may have also preferred the control over the MALB grape juice as the presence of MALB decreased the grape aroma intensity.

The control grape juice had a fresh grape aroma while the grape aroma intensity of the MALB juice was less intense. Panelist significantly preferred the aroma of the control juice significantly at 1.8 MALB/L and 3.6 MALB/L which corresponded to the aroma threshold.After evaluating the aroma of the juices, panelists were asked to evaluate the taste/flavour of the juices (Figure 8). Results showed that 57% of the panelists preferred the control juice over the MALB juice at 0.45 MALB/L, 57% of the panelists preferred the control juice over the MALB juice at 0.9 MALB/L, 60% of the panelists preferred the control juice over the MALB juice at 1.8 MALB/L, 80% of the panelists preferred the control juice over the MALB juice at 3.6 MALB/L. Based on panelists comments, the MALB juice was preferred over the control Concord grape juice due to its the intense grape flavour and less vegetal and musky flavour.

The aroma and flavour/taste preference for the MALB Concord grape juice varied. The aroma of control Concord juice was significantly preferred over MALB juice at concentrations 0.45, 1.8 and 3.6 MALB/L as compared to flavour/taste (3.6 MALB/L) ($p < 0.05$). At this concentration, the attributes associated with MALB were more intense and more noticeable and panelists preferred neither the aroma nor taste/flavour. This indicated aroma is not a good indicator of flavour intensity or aroma is more sensitive than flavour. Flavour is the sum of perceptions resulting from the stimulation of sense ends that are grouped together. Flavour is a combination of aromatics, taste and chemical feeling factors (Meilgaard et al. 2007).

Niagara grape juice

The percentage of panelists preferring the aroma and flavour of the control Niagara grape juice (No MALB) over the MALB Niagara grape juice is shown in Figure 9 and 10, respectively. In both figures, the 5% significance is indicated by the dotted line. The percentage of panelists preferring the aroma of control Niagara grape juice (0 MALB/L) over the MALB juice is represented in Figure 9. As the concentration of MALB increased from 0 MALB/L to 3.6 MALB/L, the number of panelists preferring the aroma of the control Niagara grape juice increased. At 0.45 and 0.9 MALB/L, the preference for the aroma of the control over MALB juice was evenly split at 50%. At 1.8 MALB/L, 52% of the panelists preferred the control juice over the MALB juice, and at 3.6 MALB/L, 63% of the panelists significantly preferred the control juice over the aroma of the MALB juice.

Panelists described the aroma of the control Niagara grape juice as more honey, less earthy and grassy, having a more pleasant aroma, more intense fruity aroma compared to the MALB Niagara juice. Panelists who preferred the aroma of the MALB juice described the juice as having a more intense grapey aroma, and more pleasant aroma. From the threshold study, the detection threshold was 0.68 MALB/L, however

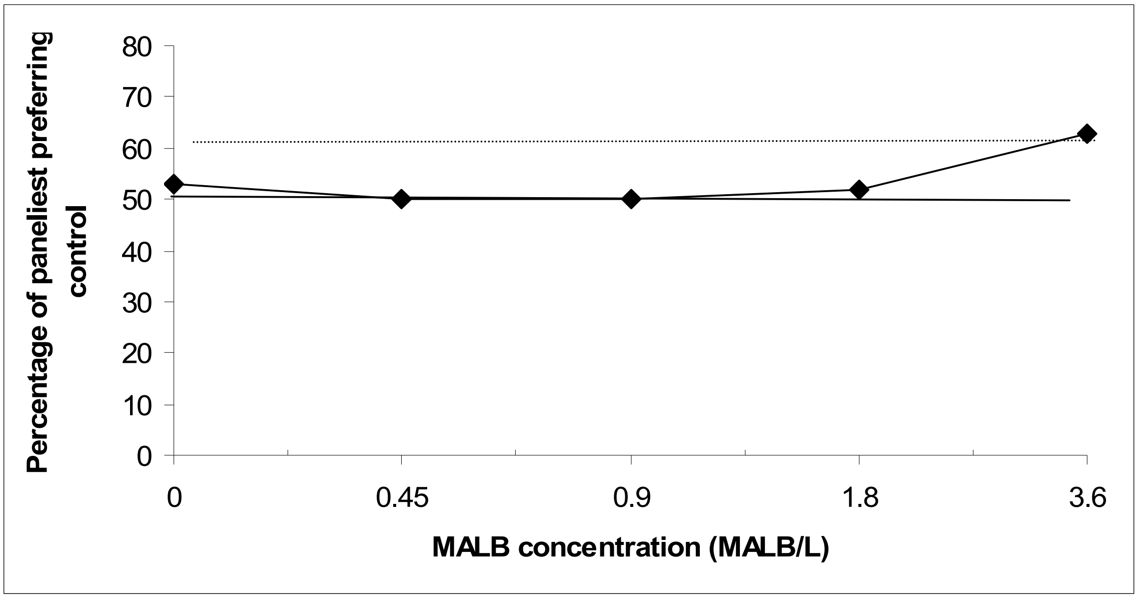

Figure 9. Percentage of panelists (n = 60) preferring the aroma of control Niagara grape juice. The solid line (50%) represents no preference and the dotted line (62%) indicates significance at $p \leq 0.05$.

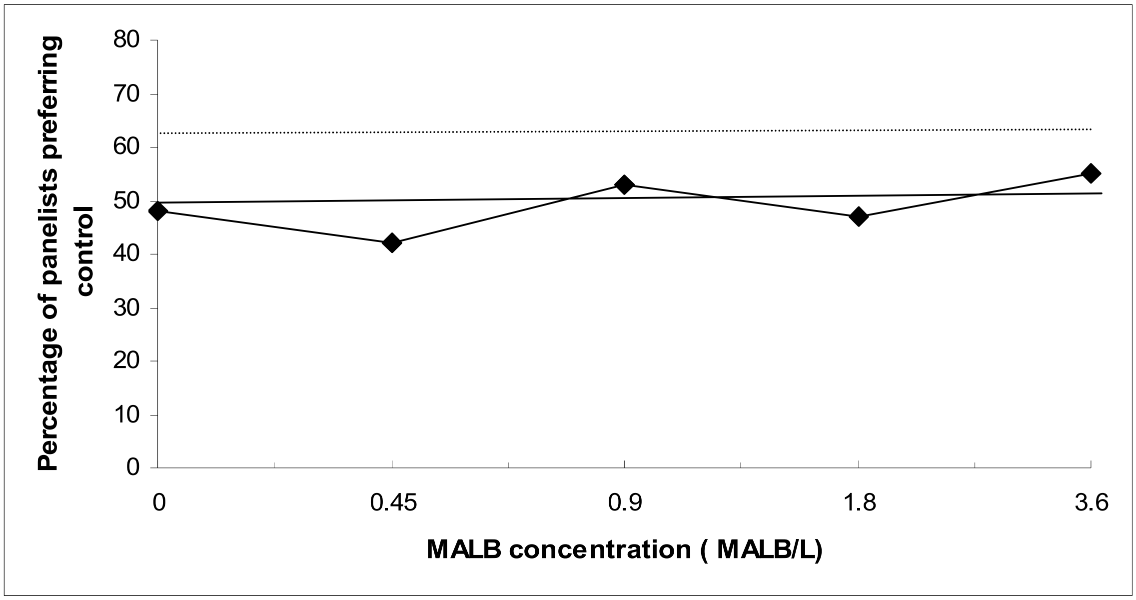

Figure 10: Percentage of panelists (n = 60) preferring the flavour of control Niagara grape juice. The solid line (50%) represents no preference and the dotted line (62%) indicates significance at $p \leq 0.05$

panelists did not express significant preference for the aroma of the MALB Niagara grape juice until 3.6 MALB/L. This indicated the panelists in the consumer rejection study may have not been as sensitive as the panelists in the aroma detection study, indicating differences in sensitivities between people.

After evaluating the aroma of the juices, panelists were asked to evaluate the taste/flavour of the juices, 42% of the panelists preferred the control juice over the MALB juice at 0.45 MALB/L, 53% of the panelists preferred the control juice over the MALB juice at 0.9 MALB/L, 47% of the panelists preferred the control juice over the MALB juice at 1.8 MALB/L, 55% of the panelists preferred the control juice over the MALB juice at 3.6 MALB/L. Panelists preferred the control Niagara grape juice at 0.9 MALB/L and 3.6 MALB/L, while the MALB Niagara grape juice was preferred at 0.45 MALB/L and 1.8 MALB/L.

However, none of these results were significant. Based on comments, panelists preferred MALB juice because it was less astringent, had a more intense grape flavour and was sweeter compared to control Niagara grape juice. Although panelists significantly preferred the aroma of the control Niagara grape juice at 3.6 MALB/L, the same result was not observed with the taste/flavour at equivalent concentration. Based on the threshold panel from the earlier study, MALB had a threshold average of 0.65 MALB/L. One may have expected that at 0.9 MALB/L, panelists would significantly prefer the control Niagara grape juice over the MALB juice. Based on the aroma values in Figure 9, did not predict flavour preference. However with the trained panel, it was observed that as MALB concentration increased there was not a consistent trend of increased attribute intensity. From the consumer study, variability among individuals is apparent as more than 50% of the panelists preferred MALB juice. However this shows the variability among individuals and their perception.

In comparison, a greater percentage of panelists significantly preferred the aroma of control Concord grape juice than of control Niagara grape juice. For Concord grape juice, significant preferences were observed with 0.45 (63%), 1.8 (77%) and 3.6 MALB/L (77%). For Niagara grape juice, the significant preference for the control was observed at 3.6 MALB/L (63%). Based on comments, the preference for the flavour of either control Niagara juice or MALB juice was based on individual preferences for sweet and sour attributes in the juice.

Consumers perceive food and beverages in the order of appearance odour/aroma, consistency and texture and flavour (Meilgaard et al. 2007). Results from the threshold panels in earlier studies showed panelists could detect MALB at low concentration in the juice. However it must not be assumed that detection can be translated to preference. This was observed with the consumer rejection studies for Concord and Niagara grape juice. The sense of smell may not be a good indicator of the intensity of flavour.

Results from this study indicated the presence of MALB affected pH, Brix and titratable acidity levels. The pH and titratable acidity values may give an indication of anticipated sourness and acidity A decrease in Brix values gave an indication of decreased sweetness. However these results did not directly translate to differences in sensory attributes as evaluated by the trained panel. Although differences in sweetness and sourness were observed in Niagara grape juice, none were significantly observed for Concord grape juice. For the aroma detection threshold, the presence of MALB was detected in Concord grape juice at a concentration of 1.98 MALB/L. However the trained panel only found vegetal aroma and flavour to be significantly different between MALB concentrations. This significance continued to the consumer rejection threshold which showed that MALB juice was significantly rejected at concentrations above the detection threshold. However, this significance did not translate into the taste/flavour component of the juice.

For Niagara juice, the aroma detection (0.65 MALB/L) was lower than that of Concord grape juice. This was also noticed with the trained panel when panelists were able to distinguish more difference in aroma, taste/flavour and mouthfeel attributes compared to Concord grape juice. However, based on the consumer rejection study, panelists only preferred the aroma of the control juice at 3.6 MALB/L while for flavour, there was no significant preference. Consumers only significantly disliked the taste/flavour of the juice at 3.6 MALB/L. When comparing the analytical results to the sensory results, it seems the most effective way to determine the presence of MALB is through sensory analysis.

CHAPTER FIVE

CONCLUSION

The objective of the research was to determine if the presence of MALB impacted the sensory properties of Niagara and Concord grape juice. The null hypothesis was rejected and the alternative was supported. As expected, the Brix, pH and TA changed with the presence of MALB. In both juices, the pH decreased as the concentration of MALB increased, which suggested the acidity of the juice increased. The Brix decreased as the concentration of MALB increased suggesting that the ratio of dissolved sugars decreased. The TA decreased which suggested the acidity and presence of organic acids decreased.

For the aroma threshold panels, as the MALB concentration increased the taint became more noticeable thus giving an average detection threshold 1.89 MALB/L in Concord grape juice and 0.65 MALB/L in Niagara grape juice. After 11 mo of storage, the aroma threshold of Concord grape juice decreased to 1.66 MALB/L; however this value was not significantly different from the other panels. Therefore, another factor which may account for the non-significant difference is the number of panelists (n = 17) used in the storage study compared to the number of panelists (n = 24) used in the repeat session. The smaller number of panelists may have increased the error terms.

Although specific trends were not observed with the trained panel, the presence of MALB affected some of the Concord and Niagara grape juice attributes. In Concord grape juice, significant differences were observed with vegetal aroma and flavour previously identified and associated with methoxypyrazine. For Niagara grape juice, although no trends were observed, honey and vegetal aroma, earthy, vegetal and honey flavours along with sweetness, sourness and astringency differed by the different MALB concentration ($p \leq 0.05$). For consumer rejection studies, it was expected panelists would significantly prefer the control Concord grape juice over the MALB juice, however, this was not observed at all concentrations. At detection threshold, 1.8 MALB/L and above panelists rejected the MALB juice based on aroma. However these results were not the same with flavour. Preference for control juice over MALB tainted juice was significant at 3.6 MALB/L, the highest concentration evaluated. In Niagara grape juice, due to the lower BET value and the significance of grape juice attributes recognized by panelists it was expected that consumer rejection threshold values would be lower than observed in Concord juice. Panelists detected the presence of MALB but did not think its presence was adequately objectionable to reject the juice. The only MALB concentration that consumers preferred less than the control Niagara juice in aroma and taste/flavour was 3.6 MALB/L. Indicating aroma was not a good indicator of flavour in MALB tainted juice. Based on consumer comments, it was suggested that the preference for MALB Niagara grape juice was based on preference for sweetness and sourness.

Overall, the presence of MALB was more easily detected in Niagara grape juice than in Concord grape juice. Reasons for this may be due to the increased concentration of methyl anthranilate in Concord grape juice and also the presence of higher phenolic compounds which may affect aroma and flavour binding interactions. From the results of this study, grape processors have an indication of the effect of the presence of MALB on the sensory properties of grape juice. Although analytical values can give a slight indication of the presence of MALB, the main contamination can be effectively tested by sensory analysis. However, processors should note aroma intensity does not give an indication of flavour intensity. They should also be aware of sensitivity differences to MALB among individuals and recognize the presence of MALB can affect Concord and Niagara grape juice differently. Grape processors may now have an indication of what to expect with small in-house difference tests. These tests can help in the determination of MALB contamination. For Concord grape juice, the increase of vegetal aroma and flavour may be used as an indication of contaminations.

In Niagara grape juice, the decision might be a bit more challenging as significant differences were observed with the trained panel, but based on the consumer rejection threshold, panelists did not significantly reject the MALB juice at concentrations lower than 3.6 MALB/L. Therefore, for Niagara grape juice, difference testing may be a better way to determine contamination.

When MALB are incorporated into the juice making process consumers can detect MALB taint aroma at a low threshold. The characteristics of the juice such as aroma, taste/flavour and mouthfeel of the juice can vary depending on MALB concentrations. This was evident with the Niagara grape juice. The presence of MALB in juice can cause consumers to reject the juice. The results of this study can be used to set tolerance levels of MALB at harvest and accidental inclusion into the grape juice making process. These limits are going to vary depending on the type of juice. Knowing the effect of MALB on the characteristics of juice can help companies during recalls. These recalls may result in financial loss and credibility to the company and growers.

FUTURE WORK

While this study addressed some important research questions, some tests could be improved in the future. Due to the intense aroma of Concord grape juice, increased rest between juices during the aroma threshold determinations may have reduced the carry-over effect. During training, allowing panelists to generate terms used to describe the MALB Concord grape juice and have a longer training period may have affected the differences observed among the MALB juices. A longer training period, a better understanding of the attributes and more feedback may have been helpful for the trained panel for Concord grape juice.

The main objective of this study was to determine the effect of MALB on grape juice. During grape juice processing, filtration was not performed for fear of removing volatiles. Research could be performed on grape juice after filtration to determine if filtration results in detection threshold changes, changes in the attributes observed by the trained panel or consumer rejection of the MALB juice. Remediation techniques to decrease or remove the distinguishing characteristics of MALB taint could be investigated. Besides filtration, it may be useful to research remediation techniques such as using activated charcoal in juice to determine if they decrease or remove MALB taint from Concord and Niagara grape juice.

In future studies threshold determination, trained panel and consumer rejection could be conducted on the grape juice after storage of grape juice from 9 -12 mo. This would determine the impact of storage on the MALB characteristics in the juice. It would be interesting to determine whether dead MALB have similar effects on the sensory properties of Concord and Niagara grape juice compared to live beetles. With gas chromatography/mass spectrometry (GC/MS), the fluid released from the MALB could be analyzed and compared to the MALB juice to determine if the compounds in the fluidare present in the juice. An olfactomer should also be used to confirm if the description of the volatiles released from the MALB fluid is similar to those emitted from the juice.

CHAPTER SIX

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