Evaluation of Sensory Thresholds and Perception of Sodium Chloride in Grape Juice and Wine

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Abstract: Poor water quality and lack of rainfall can lead to higher salt loads in vineyard soil and the production of wine with sodium chloride (NaCl) concentrations that may affect wine quality or exceed regulatory limits. Here, study 1 aimed to determine NaCl sensory thresholds in grape juice and wine so that better harvest and processing decisions could be made regarding salt-affected fruit. A whole-mouth gustatory method was used to determine detection and recognition thresholds of NaCl in water, red and white juices, and wines. The NaCl sensory thresholds were often within legal boundaries; thus, a significant proportion of wine consumers may detect salt in wines at concentrations below the legal NaCl limits. The detection and recognition thresholds of NaCl in grape juice and wine increased with panelist age. Study 2 investigated how NaCl affects wine sensory properties. Sensory evaluation using a trained descriptive analysis panel (n = 9) and chemical and elemental analyses were conducted on four Chardonnay wines made from separate vineyards where the fruit was perceived to contain varying degrees of saltiness and results were compared to Chardonnay wine samples spiked with 0.5 or 1 g/L NaCl. Wines made from fruit grown on salt-affected vines and wines spiked with NaCl had similar sensory characteristics. Salty and soapy attributes were correlated and associated with higher Na and Cl concentrations. Fruit expression was associated with wines containing less Na and Cl. When determining acceptable salt concentrations in juice and wines, winemakers need to consider sensory impacts, legal requirements, and who conducts the sensory assessment.

Key words: salinity, detection threshold, recognition threshold, salt perception, grape juice, wine

Poor water quality, climate change, drought, and water restrictions are among the factors that contribute to an increase in soil salinity and sodicity across various viticultural regions of Australia (Walker et al. 2010) and internationally (Scacco et al. 2010). There has been a significant decline in rainfall across much of southern Australia over the last 50 years (Hope et al. 2010). While the salinity of irrigation water in some regions may be relatively low (<0.6 dS/m), in other regions of southeastern Australia it can be higher (1–3 dS/m) (Walker et al. 2010). Together, this decline in rainfall and increase in salinity can have direct consequences on the uptake of salt by the roots, leaves, and fruit of grapevines, which in turn may produce wines with high sodium chloride (NaCl) concentrations. High NaCl concentrations in wine are generally not considered favorably and have been anecdotally described as salty, flat, dull, soapy, seawater-like, and brackish (Walker et al. 2010).

Salt is one of the five accepted taste qualities (sweet, sour, bitter, salty, and umami) perceived and recognized by humans (Sugita 2006). Salty tastes are believed to play a role in the maintenance of ion and water homeostasis; however, salt consumption at high concentrations can be unpleasant (Sugita 2006). The basic anatomical units of taste detection are taste-receptor cells, which are assembled into taste buds and distributed across different papillae of the tongue and palate epithelium (Sugita 2006) as well as other regions of the upper gastrointestinal and respiratory tracts.

Sodium (Na^+) and chloride (Cl^-) ions are required for the activation of the salt receptor cells (van der Klaauw and Smith 1995). The exact nature of the human salt receptor is yet to be completely determined, although it is likely to consist of a Na^+ ion channel (Chandrashekar et al. 2010). Mouse-based model systems suggest that there are two epithelial Na^+ channels, one specific for Na^+ (a positive salty taste) and activated at low concentrations. The second is activated by higher Na^+ concentrations and by cations and is responsible for the negative taste of these cations (Chandrashekar et al. 2010). Other compounds can exhibit a salty taste, the most notable for wine being KCl; however, the taste of this salt (and many others) is also bitter (van der Klaauw and Smith 1995). The pathways involved in response to Cl^- are not as well characterized. There are several classes of Cl^- channels reported to be expressed in taste-receptor cells (Sugita 2006), yet the specific functions of taste cell-associated Cl^- channels are still not well understood.

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In order to make timely decisions about the suitability of fruit for winemaking, many industry professionals are forced to rely on their sensory perception of saltiness of the grapes, particularly as few wineries have sufficient resources to test Na^+ and/or Cl^- concentrations in grape samples of all fruit before harvest. However, the reliability of tasters to accurately perceive salt in grapes, must, or wine is unknown, and preliminary observations have suggested that errors may occur. For example, when fruit is tasted by a panel of winemakers in the vineyard it can taste salty to some but not all, compromising harvest decisions. Of concern is that a perceived taste of salt in wine may lead to reduced consumer approval of and confidence in the product and/or brand. The perception may be low but the chemical analysis may be high, and thus exceed legal limits. Either way salt can threaten the quality of the product or its export capability.

The legal limit in Australia is 1 g/L soluble chloride expressed as NaCl (FSANZ 2012): the wine cannot contain >606 mg/L Cl^-, expressed as that ion. The legal limit for Na^+ and/or Cl^- varies widely among countries and can implicate either or both ions, with the lower limits set by South Africa (100 mg/L Na^+), Switzerland (60 mg/L Na^+), and Turkey (500 mg/L Cl^-, expressed as NaCl) (Stockley and Lloyd-Davies 2001). Depending on the market in which the wine will be sold, the acceptable concentration of NaCl may be low.

Established population thresholds for NaCl in grape juice and wine would help in the sensory evaluation of fruit for the presence of salt. Thresholds would also help clarify vineyard observations that some parcels of fruit taste salty although containing relatively low Na^+ or Cl^- concentrations. Detection and especially recognition thresholds in wine would help wine producers in deciding whether to release a product that, despite containing levels of Na^+ or Cl^- ions below legal limits, may be perceived as salty by a certain percentage of the target market. Numerous studies have examined NaCl taste thresholds in humans using a variety of techniques, including whole-mouth methods (Stahl 1973), but there is little information on salt taste thresholds in grape juice or wine. Thus, study 1 of the current work determined NaCl detection and recognition thresholds in white and red grape juices and wines of the three quartiles in a population of normal subjects. In addition, there are limited reports regarding salt taste in wine, and study 2 involved a detailed, objective sensory description of the perception of NaCl in wine. Wine spiked with NaCl and wines made from vineyards selected to give a range of salty tastes and Na^+ and Cl^- concentrations were evaluated to distinguish between direct (i.e., NaCl addition) and indirect (e.g., by altering fruit exposure or delaying maturity as a consequence of high salt in the soil) effects of salt on wine sensory descriptors.

Materials and Methods

Experimental design. This study had two parts. Study 1, threshold testing, was devised to estimate both the detection and recognition thresholds of NaCl in red and white wine grape juice and wine and used a whole-mouth gustatory method previously used in a clinical setting to determine thresholds for basic tastes in water (Yamauchi et al. 2002). Initially, we wanted to estimate measures of NaCl in water to confirm that this methodology worked in our laboratory by comparing our obtained threshold values with those in the literature. A large, trained panel was used for the threshold testing. Study 2 examined salt perception in wine. This study was conducted to gain an objective understanding of the sensory ramifications associated with the presence of NaCl in wine, which was derived either from the direct addition of NaCl to wine or the use of fruit with naturally high NaCl concentrations to produce wine.

Vineyard and grapes. In 2009, *Vitis vinifera* Shiraz and Chardonnay grape samples were sourced from the University of Adelaide (UA), Waite Campus, Coombe Vineyard, South Australia, to generate juice for the determination of NaCl thresholds in red and white grape juice for study 1. To examine the perception of NaCl in white wine in study 2, four commercial Chardonnay grape samples were collected; three from the Padthaway geographical indicator (GI) region and one from the Langhorne Creek GI region, Australia. These juice samples were chosen for their unique sensory qualities, in that some were perceived as tasting salty when assessed as part of the commercial field-grading process, although this perception did not consistently reflect NaCl composition.

Juice production, wine, and winemaking. Shiraz and Chardonnay grapes for study 1 were destemmed and crushed at the UA Hickinbotham Roseworthy Wine Science Laboratory winemaking facility using a combined crusher/destemmer (Enoitalia, ENO-15, Florence, Italy) and a Diemme 130 L membrane press (Diemme Enologia, Lugo, Ravenna, Italy). During crushing, 50 mg/L sulfur dioxide (SO_2) was added as a 20% solution of potassium metabisulfite (PMS) and 20 L of both unfiltered juices were stored at 0°C in 2-L glass containers prior to use for both the determination of the NaCl thresholds and elemental analysis.

Unoaked Chardonnay and Shiraz 2-L bag-in-box wines (Yalumba, Angaston, Australia) were used for the determination of NaCl thresholds in white and red wine in study 1. The same Chardonnay was used as the base wine to be spiked with NaCl in study 2.

The four parcels of commercial Chardonnay fruit were processed as per study 1 (see above) and the juices were separated into 20-L food grade plastic containers and four 2-L containers for juice analysis. The juice samples were then frozen at -20°C for later use. The 20-L sample of each of the four juices was thawed overnight at 15°C. Pectolytic enzymes (LaFazyme, Laffort, Bordeaux, France) were added at 1 g/hL to aid settling and clarification prior to fermentation. Following settling, the clear juices were siphoned into 22-L stainless-steel pressure vessels and inoculated with an active-dried form of QA23 (*Saccharomyces cerevisiae,* Lalvin, Lallemand, Montreal, Canada) at 0.25 g/L. Diammonium phosphate at 200 mg/L was added to all fermentations prior to yeast inoculation. Single ferments of each juice were carried out at ~15°C. Must sampling was conducted daily for analysis of temperature and sugar concentrations. When the ferments contained less than 2 g/L residual sugar, the wines were removed from the gross lees by siphoning the clear wine from the fermentation vessel. The clear wines were transferred into another closed, stainless-steel vessel containing 25 mg/L PMS, acid-adjusted with tartaric acid (1.2 g/L) to aid SO_2 protection and settled and cold stabilized for one month by storing at 0°C with an addition of potassium hydrogen tartrate (4 g/L). The wines were then warmed to room temperature and protein stabilized with bentonite (2 g/L). After a final racking, another addition of SO_2 was made by adding 60 mg/L PMS. The wines were filtered (nominal rating of 0.8 um) and then bottled in 375 mL green glass bottles under inert gas and sealed with roll-on tamper-evident screwcaps. Wine bottles were stored horizontally at 15°C until further chemical and sensory analyses.

Eight wines were analyzed by panelists in the descriptive analysis component of study 2. There was one replicate of each of the four commercial Chardonnay wines: PHH, Padthaway high salt perception, high Na^+ analysis; PHL, Padthaway high salt perception, low Na^+ analysis; PLL, Padthaway low salt perception, low Na^+ analysis; and LHL, Langhorne Creek high salt perception, low Na^+ analysis. There were also duplicate samples of the commercial unoaked Chardonnay spiked with either 0.5 g/L or 1 g/L NaCl (Merck Pty., Kilsyth, Victoria, Australia).

Study 1: Whole-mouth gustatory threshold testing. *NaCl thresholds in water.* NaCl detection and recognition thresholds were determined in vivo by a modified whole-mouth gustatory test (Yamauchi et al. 2002). Testing was conducted in an open plan, sensory lab with 60 individual cubicles, illuminated with a mixture of natural and fluorescent light. Subjects (n = 221; 92 females and 129 males, between 19 and 56 years) were recruited from the enology and viticulture under- and postgraduate student cohort and were all familiarized with four taste qualities: 0.2% NaCl (salt), 0.2% tartaric acid (sour), 2% sucrose (sweet), and 0.001% quinine sulfate (bitter) solutions in water. These subjects had at a minimum 20 hours of basic taste quality evaluation training, and although they had not been screened for taste blindness, all subjects could correctly identify the four taste stimuli they were required to evaluate. At the time of NaCl testing, thresholds were also determined for sucrose, monosodium glutamate, quinine sulfate, and tartaric acid (data not presented) in a random order so subjects did not know the presented target compound.

A stock solution of 12.8 g/L NaCl and seven serial 1:1 dilutions were prepared just before use in room temperature potable water that had been determined by trained sensory staff to be free of odors or taints. Water (1 mL) was applied evenly to the tongue in a circular motion using a plastic pipette. The subject then swallowed the solution and was asked to state whether they perceived a taste sensation and if they could, state the nature of the taste. Test solutions (1 mL) were applied at increasing concentrations after a 60-second interval. Each subject evaluated each of the seven NaCl solutions once. The NaCl taste detection threshold was determined to be the lowest concentration at which the subject could perceive a taste sensation, while the taste recognition threshold was the lowest concentration at which the subject could correctly identify the taste quality as salty.

NaCl detection and recognition thresholds in grape juice and wine. Subjects were volunteer enology staff and higher degree research students from the UA Waite Campus plus experienced panelists from a database maintained by the UA Waite Campus Sensory Research group (n = 122). Of the 122 subjects, 75 were male, 47 were female, between 19 and 74 years, with diverse backgrounds representing 16 different nationalities. No subject used medications known to affect taste. Not all of the 122 subjects undertook each of the four threshold tests and they had not been tested for taste blindness.

At the time of this study, there was no information on the thresholds of NaCl in grape juice and wine. NaCl was added to the Coombe vineyard Chardonnay and Shiraz juices and the unoaked Chardonnay and Shiraz 2-L bag-in-box wines at varying concentrations to produce a range of 40 samples to help gauge a suitable concentration range to examine NaCl thresholds within these media. These concentrations were chosen based on our knowledge of NaCl thresholds in water using the whole-mouth gustatory method in the current study, by threshold levels in water reported by others (Yamauchi et al. 2002), plus blind bench-top sensory assessment. The final range determined was from the highest concentration: 24 g/L serially diluted 1:1 down to 0.1 g/L. NaCl concentrations were reported including the Na^+ and Cl^- in the base wine (see below for analysis). Testing was conducted in a 12-booth sensory laboratory under fluorescent lighting. A stock solution of 24 g/L NaCl and eight serial dilutions were prepared just before use in room temperature juices and wines. Beginning with the base media, a sample (1 mL) was applied evenly to the tongue in a circular motion using a plastic pipette. The subject then swallowed the solution and was asked to state whether they perceived a taste sensation and, if they could, to state the nature of the taste. Test solutions (1 mL) were applied at increasing concentrations after a 60-second interval. Each subject was randomly allocated to a juice and wine series and evaluated each of the eight NaCl solutions within a series once; thereby each taster undertook one juice and one wine evaluation in one sitting only. The NaCl taste detection and recognition thresholds were determined as for water.

Study 2: Descriptive analyses of commercial and NaCl-spiked Chardonnay wines. A descriptive analysis (DA) was performed five months after bottling to quantitatively define differences in the sensory profiles of all eight wines: the four Chardonnays from individual commercial vineyards and the unoaked, Chardonnay bag-in-box wine spiked with NaCl at two levels (both in duplicate). Wines were evaluated over September and October 2009 by a panel of nine people (three females and six males) comprised of UA staff and students enrolled in postgraduate enology and viticulture coursework and between 28 and 43 years old. Prior to formal DA training, panelists underwent 20 hours of high-level training in aroma, taste, and trigeminal sensation detection, identification, evaluation, and ranking over a five-week period. Six of the nine panelists had previous experience with DA of wine.

Weekly two-hour sessions over six weeks and two three-hour sessions on one day in week seven were held to further train the panelists. During these training sessions, panelists were presented with 30 mL of each of the eight wines in coded, covered black INAO, 215 mL tasting glasses (to remove any possible visual cues) and instructed to individually generate and then reach panel consensus on descriptive terms. The descriptive terms ultimately agreed upon included two taste (salty and metallic), one flavor (fruit expression, where flavor is defined as aroma by mouth), and two mouthfeel (drying and soapy) attributes. Wine attributes were assessed using scale word anchors and where appropriate a sensory reference; unless stated otherwise the sensory references were made in Millipore-filtered water. For salty, the word anchors were "low intensity" comprising the bag-in-box Chardonnay wine and "high intensity" where this wine was spiked with 1.0 g/L NaCl. For metallic, the word anchors were "absent" and "low intensity" and a sensory reference was not provided. The flavor fruit expression scale was designated with word anchors "low intensity" using a reference of lemon cordial (Cottee's, Tullamarine, Victoria, Australia) diluted 1 in 20 and "high intensity" using a reference of neat lemon cordial. The scale words for the mouthfeel attribute soapy were "absent" and "very slippery" and for drying were "absent" and "very drying" and sensory references were not provided.

Panelists practiced rating the wines using an unstructured 15 cm line scale with indented end anchor points placed at 10% and 90% of the scale and a midline anchor point. Rating took place in isolated booths in a 12-booth sensory laboratory under fluorescent light and under conditions identical to those used in the subsequent formal tasting session. Intensity rating standards for fruit expression and salt were provided at each session as fruit and salt intensity rating aids. No other reference standards were provided for taste and mouthfeel, but panelists had extensive training in these attributes. Panel performance was evaluated in the last two training sessions by having each panelist assess a subsample of the wines in duplicate. These data were analyzed using PanelCheck (Nofima Mat and DTU, Informatics and Mathematical Modeling, Tromsø, Norway) and SENPAQ (ver. 5.01, Qi Statistics, Berkshire, UK). As no significant panelist by sample interactions were found, the panel was justified in beginning final evaluation of the samples.

A final discussion session outlining what would occur during formal evaluation of samples and negotiating formal session times was followed by two two-hour formal rating sessions conducted in a temperature controlled (22°C) sensory lab with 12 individual booths under fluorescent light. At each rating session, each panelist was presented with eight wines (four wines from commercial vineyards and duplicate samples of bag-in-box wine spiked with either 0.5 g/L or 1.0 g/L NaCl). Each wine was evaluated in a randomized presentation order-balanced for carryover effects and in duplicate over the course of the formal rating period. The series of 30 mL wine samples were presented in coded, black INAO 215 mL tasting glasses covered with small Petri dishes. Distilled water and unsalted crackers were provided for palate cleansing and panelists were forced to have a one-minute rest between each sample. At the beginning of each session, panelists familiarized themselves with the intensity standards and had free access to the standards outside their booths during the rating, if needed. FIZZ software (ver. 2.1; Biosystèmes, Couternon, France) was used for collection of the DA rating data.

Juice and wine chemical analyses. For both studies, the level of juice total soluble solids was measured as degrees Brix using a DMA 35N Density Meter (Anton Paar GmbH, Graz, Austria). Juice and wine samples were analyzed directly for pH and titratable acidity (TA, g/L) was measured using titration to pH 8.2 (Iland et al. 2004). Each wine was also sampled and chemically analyzed for free and total SO_2 (mg/L), volatile acidity (VA, g/L), and residual sugar (RS, g/L) by the Rebelein method (Iland et al. 2004). Alcohol content (% v/v) was determined using an Alcolyser Wine (Anton Paar) at room temperature.

The juice and wine samples were diluted 1:10 with Milli-Q water prior to analysis for concentrations of potassium (K^+) and Na^+. The juice and wines were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP–OES) (model Optima 2100DV, Perkin Elmer, Waltham, MA) by the method of Wheal et al. (2011). Samples for Cl^- analysis in both juice and wine were extracted using warm 4% nitric acid and hydrogen peroxide in 50 mL polypropylene tubes with lids on to prevent contamination on a programmable digestion system. Sample solutions were analyzed using ICP-OES following the method of Wheal and Palmer (2010).

Statistical analyses. The wine chemical composition data were analyzed with one-way ANOVA with Fisher's LSD post-hoc test using SPSS 15.0 (SPSS Inc., Chicago, IL) and Excel 2003 (Microsoft, Redmond, WA). The log normal parametric model was fitted to the threshold data for NaCl in water, grape juice, and wines. A log normal survival model was fitted to the data for each threshold response using interval censoring; for example, a subject who failed to detect the salt at a concentration of 0.12 g/L but detected it at 0.22 g/L was assumed to have a threshold detection level between 0.12 and 0.22 g/L. The model parameters were estimated by maximum likelihood estimation and a Chi-squared test was conducted between age groups using MINITAB (ver. 16; Minitab, State College, PA). For the DA, a mixed-model two-way ANOVA with assessors as random and samples as fixed factor effects was used, with Tukey's post-hoc test where $p < 0.05$ was considered significant using SENPAQ and PanelCheck. Principal component analysis was performed in XLSTAT (ver. 2009 1.01; Addinsoft, Paris, France).

Results

Juice and wine composition. Standard chemical measures of juices (Table 1) and wines (Table 2) for study 1 threshold testing and study 2 descriptive analyses were completed. All wines would be considered technically dry as the residual sugar (RS) measures were all below sweet recognition levels. Titratable acidity (TA) concentrations of the Chardonnay wines ranged from 4.9 to 6.5 g/L while that of the Shiraz wine was marginally higher at 7.2 g/L (Table 2). pH levels of all wines were typical of those desired for microbiological stability and ranged between pH 3.17 to 3.55. With the exception of the Shiraz bag-in-box wine, which contained

30 g/L free SO_2, all other wines had free SO_2 levels below 21 g/L; overall it was considered that this parameter would consequently have no bearing on sensory evaluation. Alcohol levels were lower in the wines made from Padthaway and Langhorne Creek as the fruit for these wines was collected before commercial harvest.

Juice and wine elemental analyses. There was a large contrast in ion composition of the grapes and wines selected from the different sites and in the ratios of the ions (Table 1, Table 2). When high concentrations of Na^+ and Cl^- were present in the juice the same was also seen in the wine; there was an increase in Na^+ between the juice and the wine, most likely due to the release of Na^+ from the bentonite (Catarino et al. 2008). K^+ varied between juice and the corresponding wine sample, which was likely due to the net effect of the addition of potassium metabisulfite, potassium hydrogen tartrate, and tartrate precipitation during cold stabilization. The concentration of Na^+ and Cl^- in the base wine that was spiked with NaCl was low relative to the samples from the commercial vineyards (Table 2). All of the wines met the Australian maximum residue limit of 606 mg/L Cl; however, all of the Chardonnays (including the commercial wine) exceeded the Swiss standard of 60 mg/L Na.

Detection and recognition thresholds of NaCl: Study 1. NaCl detection and recognition thresholds for the first, third, and inter quartiles were calculated from the whole-mouth gustatory threshold testing sensory results (Table 3). The population median NaCl detection threshold value was 0.31 g/L while the median recognition threshold value was higher as expected at 0.80 g/L. The first quartile could detect NaCl in water at a concentration as low as 0.14 g/L, and the NaCl recognition threshold was higher as expected and ranged from 0.36 to 1.78 g/L for the entire sample.

The median detection and recognition thresholds of NaCl in white grape juices and wines were very similar (Table 4). Interestingly, both median detection and recognition thresholds were markedly higher in red juice relative to the other base media. Chi-squared tests of the lognormal curves revealed significant differences between the different age groups for all juice and wine base media ($p < 0.05$) except for white juice, red juice, and red wine detection thresholds (Table 5). Notable increases in the median thresholds estimates were seen for the over 50 age group.

Descriptive analysis of salt-affected Chardonnay wines: Study 2. The current study evaluated the sensory effect that different NaCl additions made to Chardonnay wines and assessed the sensory characteristics of Chardonnay wines produced from commercial fruit perceived by industry

Table 3 Median estimates and quartile (lower and upper) 95% confidence intervals of NaCl salt taste detection and recognition thresholds in water (n = 221).

	NaCl detection g/L (mM)	NaCl recognition g/L (mM)
Median	0.31 (5.31)	0.80 (13.71)
First quartile	0.14–0.19 (2.40–3.26)	0.36–0.48 (6.17–8.23)
Interquartile	0.35–0.50 (6.00–8.56)	0.95–1.33 (16.28–22.80)
Third quartile	0.50–0.67 (8.57–11.48)	1.33–1.78 (22.80–30.51)

Table 1 Initial must analysis results for the Shiraz and Chardonnay juice for threshold testing (study 1) and the four commercial Chardonnay juice samples from Padthaway and Langhorne Creek (Australia) for descriptive analysis (study 2).

Must[a]	Salt perception	Na^+ analysis	Total soluble solids (Brix)	Titratable acidity (g/L)	pH	SO_2 (mg/L) free	SO_2 (mg/L) total	Element (mg/L) K^+	Element (mg/L) Na^+	Element (mg/L) Cl^-
Waite Shiraz	–	–	25.6	5.3	3.54	6	16	770	580	500
Waite Chardonnay	–	–	19.8	7.2	3.10	8	43	580	12	<7[b]
PHH	High	High	20.5	5.6	3.42	24	75	571	150	139
PHL	High	Low	21.1	7.3	3.59	10	72	410	60	145
PLL	Low	Low	25.0	5.1	3.62	16	96	1023	17	72
LHL	High	Low	17.1	4.7	3.63	11	98	1433	82	230

[a]PHH, Padthaway high salt perception, high Na^+ analysis; PHL, Padthaway high salt perception, low Na^+ analysis; PLL, Padthaway low salt perception, low Na^+ analysis; and LHL, Langhorne Creek high salt perception, low Na^+ analysis.
[b]< indicates that the result is less than the limit of detection of determination of the method.

Table 2 Chemical analyses of the control bag-in-box (BB) wines for threshold testing (study 1) and the 2009 Padthaway and Langhorne Creek Chardonnay wines for descriptive analysis (study 2).

Wine[a]	Residual sugar (g/L)	Titratable acidity (g/L)	pH	Alcohol (%v/v)	Free SO_2 (g/L)	Total SO_2 (g/L)	Element (mg/L) K^+	Element (mg/L) Na^+	Element (mg/L) Cl^-
Shiraz BB	0.3	7.2	3.35	13.5	30	162	1080	39	58
Chardonnay BB	0.2	6.5	3.17	12.2	21	158	720	74	65
PHH	1.4	6.3	3.30	11.8	11	122	470	330	144
PHL	0.7	5.1	3.46	11.8	14	122	600	210	159
PLL	1.4	5.0	3.55	11.5	10	130	640	116	70
LHL	0.1	4.9	3.46	9.7	14	150	690	196	230

[a]PHH, Padthaway high salt perception, high Na^+ analysis; PHL, Padthaway high salt perception, low Na^+ analysis; PLL, Padthaway low salt perception, low Na^+ analysis; and LHL, Langhorne Creek high salt perception, low Na^+ analysis.

Table 4 Median estimates and quartile (lower and upper) 95% confidence intervals of NaCl salt taste detection and recognition thresholds of subjects categorized by grape juice and wine.

	NaCl detection threshold g/L (mM)				NaCl recognition threshold g/L (mM)			
	White juice (n = 79)	Red juice (n = 98)	White wine (n = 95)	Red wine (n = 81)	White juice (n = 74)	Red juice (n = 82)	White wine (n = 74)	Red wine (n = 76)
Median	0.22 (3.77)	1.25 (21.43)	0.30 (5.31)	0.31 (5.31)	1.53 (26.22)	2.55 (43.63)	2.05 (35.14)	1.77 (30.34)
First quartile	0.05–0.13 (0.86–2.23)	0.69–0.96 (11.83–16.45)	0.08–0.18 (1.37–3.09)	0.10–0.20 (1.71–3.43)	0.51–0.96 (8.74–16.45)	1.38–1.92 (23.65–32.91)	0.83–1.37 (14.23–23.48)	0.76–1.21 (13.03–20.74)
Interquartile	0.35–0.77 (6.00–13.20)	0.81–1.37 (13.89–23.48)	0.44–0.86 (7.54–14.74)	0.38–0.73 (6.51–12.51)	1.90–3.75 (32.57–64.28)	1.89–3.00 (32.39–51.42)	2.14–3.86 (36.68–66.16)	1.74–3.04 (29.82–52.11)
Third quartile	0.41–0.87 (7.03–14.91)	1.66–2.21 (28.45–37.88)	0.54–1.00 (9.26–17.14)	0.50–0.89 (8.57–15.25)	2.49–4.55 (42.68–77.99)	3.39–4.73 (58.10–81.07)	3.07–5.06 (52.62–86.73)	2.59–4.11 (44.39–70.45)

Table 5 Median threshold estimates g/L (mM) by age group with 95% confidence intervals (lower and upper).

Base media	Age group	n	Median g/L (mM)	Lower 95% CI g/L (mM)	Upper 95% CI g/L (mM)
White juice detection	18–21	15	0.30 (5.11)	0.21 (3.51)	0.43 (7.42)
($p < 0.714$)	22–25	16	0.15 (2.52)	0.06 (1.05)	0.35 (6.03)
	26–30	14	0.30 (5.21)	0.16 (2.74)	0.58 (9.94)
	31–49	22	0.16 (2.74)	0.08 (1.34)	0.33 (5.67)
	50+	12	0.18 (3.12)	0.05 (0.82)	0.68 (11.66)
White juice recognition	18–21	15	0.81 (13.95)	0.36 (6.15)	1.85 (31.64)
($p < 0.007$)[a]	22–25	14	1.15 (19.64)	0.60 (10.22)	2.20 (37.78)
	26–30	12	3.20 (54.81)	1.55 (26.62)	6.58 (112.85)
	31–49	22	1.03 (17.71)	0.58 (10.01)	1.84 (31.52)
	50+	11	3.66 (62.72)	2.11 (36.11)	6.35 (108.87)
White wine detection	18–21	9	0.13 (2.23)	0.04 (0.60)	0.48 (8.21)
($p < 0.015$)	22–25	24	0.26 (4.49)	0.16 (2.81)	0.42 (7.22)
	26–30	20	0.24 (4.18)	0.16 (2.76)	0.37 (6.34)
	31–49	29	0.37 (6.29)	0.20 (3.48)	0.66 (11.38)
	50+	13	0.66 (11.26)	0.26 (4.42)	1.67 (28.68)
White wine recognition	18–21	9	1.53 (26.22)	0.69 (11.83)	3.40 (58.22)
($p < 0.008$)	22–25	18	2.67 (45.75)	1.91 (32.69)	3.73 (64.00)
	26–30	14	1.53 (26.14)	0.86 (14.65)	2.72 (46.62)
	31–49	21	1.40 (23.94)	0.87 (14.88)	2.25 (38.51)
	50+	12	4.77 (81.69)	3.00 (51.49)	7.56 (129.61)
Red juice detection	18–21	9	1.02 (17.40)	0.96 (16.39)	1.08 (18.46)
($p < 0.146$)	22–25	25	1.05 (18.00)	0.80 (13.75)	1.38 (23.57)
	26–30	22	1.37 (23.46)	1.03 (17.71)	1.81 (31.07)
	31–49	29	1.25 (21.39)	0.91 (15.60)	1.71 (29.34)
	50+	13	1.54 (26.41)	0.98 (16.71)	2.44 (41.75)
Red juice recognition	18–21	8	2.29 (39.32)	1.30 (22.21)	4.06 (69.61)
($p < 0.0001$)	22–25	19	2.24 (38.43)	1.81 (30.99)	2.78 (47.65)
	26–30	18	3.28 (56.17)	2.35 (40.23)	4.58 (78.42)
	31–49	25	1.91 (32.75)	1.45 (24.82)	2.52 (43.26)
	50+	12	5.07 (86.90)	3.20 (54.80)	8.04 (137.81)
Red wine detection	18–21	18	0.30 (5.18)	0.20 (3.39)	0.46 (7.95)
($p < 0.341$)	22–25	14	0.21 (3.51)	0.16 (2.66)	0.27 (4.64)
	26–30	15	0.27 (4.63)	0.08 (1.31)	0.95 (16.25)
	31–49	22	0.39 (6.60)	0.22 (3.77)	0.68 (11.59)
	50+	12	0.33 (5.69)	0.24 (4.03)	0.47 (8.04)
Red wine recognition	18–21	18	1.34 (23.04)	0.82 (14.11)	2.20 (37.64)
($p < 0.003$)	22–25	11	1.65 (28.32)	0.77 (13.20)	3.54 (60.68)
	26–30	14	2.75 (47.19)	1.10 (18.77)	6.92 (118.63)
	31–49	22	1.43 (24.46)	0.88 (15.05)	2.32 (39.78)
	50+	11	5.04 (86.37)	1.89 (32.34)	13.46 (230.70)

[a] $p < 0.05$ was significant between the 50+ age group and all other age groups within a base media by Chi-squared tests.

professionals as possessing different levels of salt taste and covering a range of Na^+ and Cl^- concentrations. In a test of each assessor's repeatability against the panel average repeatability, no significant *p* values were obtained for any attribute, indicating good performance. In both sessions, panelists were able to discriminate the eight wines with the exception of the replicates for the 1.0 and 0.5 g/L spiked wines, as expected.

The sensory attribute means were analyzed using principal component analysis (PCA) and the resulting plot overlaid with Na^+, K^+, and Cl^- ion mean concentrations as supplementary data: that is, these were not included in the mathematical analysis (Figure 1). PCA explained 87% of the variation in the data between the wine samples within the sensory space. The first principal component (PC) accounted for 66% of the variation. Salty and soapy attributes were highly correlated and diametrically opposed to the fruit expression along PC1, while drying contributed strongly to PC2.

The NaCl addition had a significant effect on wine sensory attributes and the wines with the 0.5 and 1.0 g/L NaCl additions had incrementally less fruit expression and more salty taste and soapy (viscous) mouthfeel. The PHH and LHL wines clustered with the commercial wine with 1 g/L of added NaCl, despite containing substantially less Na^+ (Table 2). The PLL wine was similar in attributes to the 0.5 g/L NaCl wine, being less salty and higher in fruit expression than the other four wines, while PHL was intermediary in these attributes. PC2 accounted for 21% of the variation and was strongly driven by drying characters, concomitant with lower K^+ and Cl^- ion concentration. The PHH wine was more drying than all wines, while 0.5 g/L was the least drying.

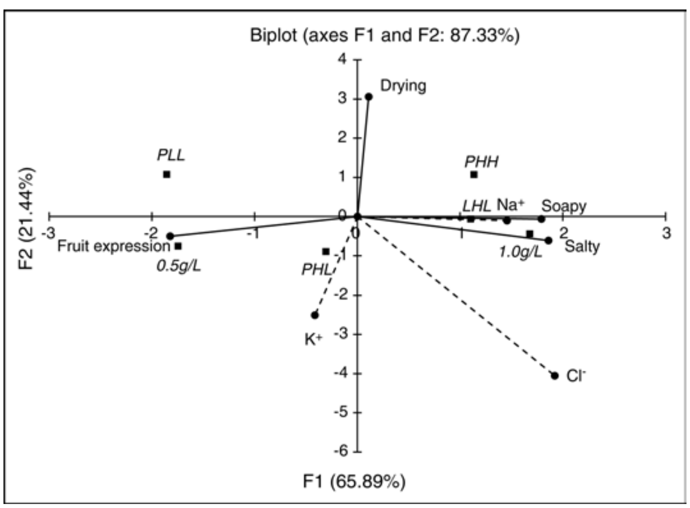

Figure 1 PCA biplot of the means of sensory attributes (solid vectors) and ions as supplementary data (dashed vectors) for Chardonnay wines (solid square data points). 0.5 g/L and 1.0 g/L represent means of two replicates each of the Chardonnay bag-in-box base wine with the addition of 0.5 g/L and 1.0 g/L NaCl. Chardonnay wines made from juices perceived to have different salt taste intensity: PHH, Padthaway high salt perception, high Na^+ analysis; PHL, Padthaway high salt perception, low Na^+ analysis; PLL, Padthaway low salt perception, low Na^+ analysis; and LHL, Langhorne Creek high salt perception, low Na^+ analysis.

Discussion

Threshold measurements are useful for providing information on the biological potency of a taste compound within a food or beverage. The quantification of the detection and recognition thresholds of NaCl in both grape juice and wine and the development of a more clearly defined impact of NaCl on wine sensory properties will assist the wine industry. The determination of detection and recognition thresholds of NaCl in water provides a benchmark for studies further validating the whole-mouth gustatory method, while an analysis of the effect of age on sensory perception of NaCl in grape juice and wines acts as confirmation of the effects that are observed in water.

NaCl thresholds in water. The median detection and recognition thresholds of NaCl in water for the population of 221 subjects (age range 19 to 56) were 5.31 and 13.71 mM, respectively (Table 3). The recognition threshold, where the salty taste quality becomes apparent, was higher and similar to the value of 9.68 mM found elsewhere (Hatae et al. 2009). A mean NaCl taste detection threshold of 5.81 mM in seventy 12- to 13-year-old Koreans using a three-alternative forced-choice procedure was reported (Kim and Lee 2009). A study using a filter paper disk method reported a mean salt recognition threshold of 7.56 mM for 69 males and females (age range 18 to 36) (Sato et al. 2002). Wise and Breslin (2013) reported geometric mean NaCl detection (modified staircase) and recognition (modified Harris-Kalmus) thresholds of 1.6 and 14.2 mM, respectively, for 22 nonsmoking adults (age range 21 to 52). Threshold values of the current research not only reflect findings of the above studies but also approximate the values of reported detection and recognition threshold means of 13.0 and 32.0 mM, respectively, for a sample of healthy young Japanese women and men using the same sensory assessment method (Yamauchi et al. 2002). The values from our study could be slightly lower than the previously reported means (Yamauchi et al. 2002), as we used subjects who had extensive training in sensory evaluation of tastes in water. A decrease in the detection threshold due to taster familiarity or training has been seen in other studies (McBride and Laing 1979). Overall, across a range of studies and assessment methods, there was a median value detection threshold of 10 mM (Stahl 1973), which is also above what we have observed; however, our results are well within the observed ranges of 3 to 20 mM.

The thresholds were reported here as median values in order to reduce the influence of outliers, which could also partly explain the difference in the reported thresholds relative to some other studies. The rationale of using the whole-mouth gustatory method was two-fold. First, it required the taster to swallow the entire sample, ensuring access of the stimuli to all loci of the taste receptors. Second, it offered an ease of application in determining the thresholds of industry personnel and potential panelists for sensory research. While the modified whole-mouth gustatory test method involved dropping a 1 mL aliquot of solution into the mouth rather than sipping the solution or application with a filter paper, these results benchmark well with other research (Yamauchi et al. 2002).

NaCl thresholds in juice and wine. It is common practice for grapegrowers and winemakers to conduct periodic sensory evaluation of winegrapes in the time up to harvest (Olarte Mantilla et al. 2012). Fruit is assessed for flavor and/or phenolic ripeness (in addition to standard chemical measures) in order to make harvest decisions or to allocate the fruit to specific wine products or styles. Evaluation of berries in the vineyard, in contrast to sophisticated chemical and biochemical techniques, is a faster and less expensive method to assess concerns such as disease or the presence of other undesirable characters, including salt.

In white juice, the median detection and recognition thresholds were 220 and 1530 mg/L NaCl, respectively (Table 4). These values were lower than those for red juices and align well with the differences observed in the field (P.R. Petrie, author's personal observations, 2012). The median grape juice NaCl salt taste recognition thresholds are above the Australian standard of 1 g/L soluble chloride expressed as NaCl. If sensory assessment is the only parameter used to determine NaCl concentrations, the likelihood of harvesting grapes that will produce wine with illegally high NaCl concentrations increases. Further difficulties may arise with grapes produced for export markets, with even the lowest median detection threshold (220 mg/L NaCl or 82.6 mg/L Na^+ in white juice; Table 4) still exceeding the Na^+ concentration limit for Switzerland (60 mg/L Na), and the recognition thresholds in both red and white juice were well above the limits of several countries.

While the detection and recognition thresholds in red juice exceed those of white juice, extra Cl^- is typically extracted from the skins of red grapes during winemaking, with the concentration of Cl^- increasing by an average of 1.7-fold from the grape juice to the final wine (Walker et al. 2010). Red juice contains a higher quantity and greater variety of compounds, especially anthocyanins and phenolic, than white juice. Even when very complex model mixtures are used as part of sensory trials, the impact of other compounds as masking agents is generally additive (Stevens and Traverzo 1997). It is likely this occurs in the more complex matrix of the red juice, thus increasing the detection and recognition thresholds.

During the study 1 sensory analysis, the tasting panelists were not informed that the compound they were testing was salt, as the panel sessions were interspersed with sessions focusing on other taste stimuli to reduce the likelihood that the panelists would guess the target compound. When NaCl solutions were quantitatively described, distinct sensory differences were reliably described at below the recognition threshold (O'Mahony 1973). Wine experts (and potentially winemakers) are more likely to show higher sensitivity to propylthiouracil (PROP) bitterness and with this a more acute ability to perceive other taste modalities including salt (Hayes and Keast 2011). Furthermore, grapegrowers and wine producers would be aware of the symptoms of salt damage, including marginal leaf necrosis, inky black staining in the center of the leaf, and, at high levels, defoliation. In combination, these latter two factors suggest that the likelihood grapes could be processed which exceed the Australian standards for NaCl based only on sensory assessments is less than is suggested by the detection and recognition thresholds measured in this study. However, fruit could easily be processed that exceeds the standards of a range of other countries.

The median detection and recognition thresholds for salty taste were lower in red wine than in red juice; the opposite occurred for white wine, which had increased thresholds than white juice (Table 4). The decrease in the detection and recognition thresholds for red wine relative to red juice was probably due to the replacement of sugar with alcohol. Stevens and Traverzo (1997) investigated the masking of NaCl taste with sucrose or citric acid: individually each compound increased the NaCl detection thresholds by three to four times but in combination they raised the detection threshold of the NaCl by nine times. Alcohol potentially also has a masking effect; however, the sensory threshold of sucrose (in beer) is about six times that of ethanol (Meilgaard 1993) and over half of the sugar (by weight) is lost as CO_2 during fermentation, so this effect is likely to be far smaller. The red juice used in the threshold testing contained relatively high concentrations of many of the metal ions (data not presented) and Cl^-. While the concentration of the Na^+ and Cl^- was accounted for when the thresholds were calculated, the other ions may also have impacted on the taste thresholds of the juice (van der Klaauw and Smith 1995). The increase in the NaCl detection and recognition thresholds between the white juice and wine was surprising; as with the red wine we would have expected a reduction in sugar concentration to result in increased perceived saltiness. Direct comparisons between the juices and wines in the threshold portion of this study are difficult to make, as the samples were drawn from separate sources (Waite Vineyard and Yalumba Wine Company, respectively) and may have contained other compounds (acids or phenolics) that enhanced or masked the salty flavors. These threshold measures have not been extensively studied in juice or wine, but in one study the addition of low levels (subdetection threshold) of acid (rice vinegar) increased the NaCl detection threshold of solutions (Hatae et al. 2009). A review article (Breslin 1996) thoroughly summarized binary taste interactions. Research generally indicates that salts and acids enhance each other at moderate concentrations, but in contrast, salts and acids suppress each other at higher concentrations, with a notable exception: tartaric acid lowers the threshold for NaCl. Here, the Chardonnay juice used for study 1 was harvested relatively unripe compared with typical harvest Brix levels and consequently would have had correspondingly higher tartaric acid concentrations.

Similar to reports in water (Stevens and Cain 1993), we noted a significant increase in the detection thresholds of NaCl in juice and wine with age. The detection and recognition thresholds (for all but the white and red juice and red wine media) for older subjects (50+ years) was 2.5 times greater than for the younger subjects (<50 years) (Table 5), which is in the range (2 to 9 times) reported elsewhere (Stevens and Cain 1993). The influence of age on detection and recognition thresholds in grape juice and wine has the potential to impact on decisions made during the winemaking process, especially as the responsibility for accepting salt-affected fruit and final assessment of wine prebottling is often with the more experienced and therefore older winemakers. It would improve confidence in decision-making if winemakers determined their own sensory thresholds for NaCl, especially if they are potentially working with salt-affected fruit.

Impact of NaCl on wine sensory properties. A key impetus of this study was to examine the impact of NaCl on a wine's sensory properties. We were interested in the sensory impact of NaCl at levels that were generally under the legal limits in Australia and many other countries. Experienced tasters were used in our threshold experiments with the assumption that if these tasters did not detect or recognize NaCl salt taste in the base white or red wine, then it would be likely that wine consumers would not either, as training generally reduces sensory threshold concentrations (McBride and Laing 1979). Our findings indicate that the entire population of experienced tasters in this study could detect the presence of NaCl in a single white or red wine at a level below the stipulated maximum legal limit in Australia of 1 g/L soluble chloride expressed as NaCl. Of more concern was that up to 25% of these tasters could recognize the salt taste quality within a concentration range that encompassed the Australian legal limits. This potentially means wine consumers not only could perceive a difference in a wine with and without NaCl at levels below legal limits but also could recognize the wine as being salty.

In a solution of water, low concentrations of NaCl were described as sour, flat, smooth, and then sweet as the concentration of NaCl increased before the salt character was clearly identified (O'Mahony 1973). Perceived intensities of sweetness, bitterness, and sourness were ranked as weaker, and generally flavor was suppressed in bottled water solutions with the presence of suprathreshold concentrations of NaCl (Kemp and Beauchamp 1994). In experimental wines that were produced from a range of saline irrigation or rootstock trials, there was a strong linear relationship between mean salty taste score and Na^+, Cl^-, and K^{2+} concentrations in the wine (Walker et al. 2003). Tasters described the wines as having soapy characters and relatively low perceived acidity, fruit flavor, and astringency, but a formal assessment of these characters was not reported. Since the treated vines were grown under saline conditions, the direct sensory impact on wine due solely to NaCl could not be distinguished from those secondary impacts on wine sensory parameters arising from factors such as increased fruit exposure, which is a symptom of high levels of NaCl (Walker et al. 2000, 2003) and can potentially influence wine sensory properties.

The PCA plot demonstrated a strong correlation between Na+ and Cl^- concentrations with salty taste for all of the Chardonnay wines (Figure 1). Fruit expression was negatively correlated with salty taste, soapy mouthfeel, Na^+, and Cl^- levels, and occurred both in the wines that were made from grapes grown in salty conditions and in the wines spiked with NaCl. In a similar manner to previously observed trends (Walker et al. 2003), Na^+ concentration was strongly correlated to salty and soapy tastes. These results confirm that the flavor modification is directly due to NaCl, as opposed to being a secondary effect of changes in vine growth or morphology. An additional significant sensory attribute differentiating the Chardonnay wines was drying. Wines higher in this attribute also had lower levels of K^+.

The LHL wine and to a lesser extent the PHL wine clustered with the PHH wine and the commercial wine that was spiked with 1 g/L, although these wines (and the fruit) did not contain as high a concentration of Na. This finding highlights our lack of understanding of the different ions and how they interact to create the salty taste (Sugita 2006). It also highlights that care needs to be taken when evaluating the suitability of fruit for winemaking that is potentially contaminated with salt. Fruit that tastes salty may only contain moderate Na^+ concentrations that meet many international regulatory limits. Further research into how the different ions interact to give the salty taste in a range of matrixes would be valuable.

Until further studies are conducted on wine consumer rejection and acceptability of wines containing levels of NaCl below the recognition threshold, we cannot be confident that the effects would be perceived by consumers as detrimental. NaCl is often a positive sensory stimulus and a higher NaCl concentration has been shown to improve the sensory preference for a great range of products (Liem et al. 2011). Red wine made from vines grown in more saline conditions (Scacco et al. 2010) has shown positive sensory characters compared to the less salty control wines. However, wines containing 0.5 to 1.0 g/L NaCl were considered more salty, soapy, drying, and with lower fruit expression. A large segment of consumers have been shown to prefer fruitier Chardonnay wines (Lesschaeve et al. 2012), making it likely that sensory attributes of NaCl would not be perceived as desirable. Consumers may also react negatively if some regions or brands are highlighted as containing high levels of NaCl; while the health risk is low, similar concerns have been seen with the listing of processing aids on wine labels (Weber et al. 2007). These areas deserve more research.

Conclusions

This work has defined population thresholds for the concentration of NaCl in grape juice and wine. The sensory thresholds are often above the regulatory limits for salt in many countries, so care must be taken even if no salt flavors are detected in the fruit. The presence of NaCl in Chardonnay wine had a negative impact on sensory characters, even at concentrations below the recognition threshold, and appeared to be similar if the NaCl was added directly to the wine or accumulated by the vines. Knowledge of the thresholds and sensory characters of NaCl will aid with making better decisions around the harvesting and processing of potentially affected fruit, especially if winemakers are aware of their own personal thresholds.

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