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Free amino acid profiles from 'Pinot noir' grapes are influenced by vine N-status and sample preparation method

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ABSTRACT

This study examined the impact of extraction method on ammonia, free amino acids, and yeast assimilable nitrogen (YAN) concentrations in 'Pinot noir' berries obtained from a vine nutrition study (altered supply of N, P, or K). Berries were either juiced or exhaustively extracted as whole berries prior to analysis. Extracts, compared to juice samples, had a significantly higher level of ammonia–N, assimilable amino acid–N, and YAN. For example, juice YAN values were approximately 50% of extract YAN values, when both were expressed in the same units. Free amino acid profiles and relative concentrations of individual amino acids were different in juice versus extracts, depending on how well the skin fraction was extracted prior to analysis. Lowering N supply reduced free amino acids, with arginine being reduced more than the other 20 free amino acids identified in 'Pinot noir' berries. This was true in both juice and extracts. Since berry skin contributed to actual YAN, wineries that determine YAN from mainly the pulp fraction (juice) may underestimate YAN and as a result add more (artificial) N supplement than is required for the healthy fermentation of red winemaking (whole berry fermentations). Extraction procedure should be taken into consideration when comparing grape YAN.

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1. Introduction

Free amino acids and ammonia make up the majority of nitrogen (N) containing compounds that are important in wine grapes for successful alcohol and/or malolactic fermentations (Bely, Sablayrolles, & Barre, 1990). N content can be manipulated, intentionally or otherwise, by numerous viticultural and oenological factors (Bell & Henschke, 2005, references there in). Free amino acid concentrations and their profiles within grapes, can vary depending on cultivar, rootstock/scion combinations, vine nutrient management, vineyard site, and growing season, (Bell & Henschke, 2005, references there in; Gump, Zoecklein, Fugelsang, & Whiton, 2002; Huang & Ough, 1991; Rodriguez-Lovelle & Gaudillere, 2002; Spayd & Andersen-Bagge, 1996; Treeby, Holzapfel, Walker, & Nicholas, 1998).

Winemaking fermentation variables, including yeast strain, must vitamin status, sugar levels, ammonia concentration, individual free amino acids, grape maturity, must pH, titratable acidity, and even fermentation temperature will alter the yeast assimilable nitrogen (YAN) requirements of a must (Arias-Gil, Garde-Cerdan, & Ancin-Azpilicueta, 2007; Bell & Henschke, 2005, references there in; Bisson & Butzke, 2000; Gardner, Poole, & Jiranek, 2002; Mendes-Ferreira, Mendes-Faia, & Leao, 2004; Miller, Wolff, Bisson, & Ebeler, 2007; Stines et al., 2000; Ugliano et al., 2009). A moderate level of N (~125–225 mg N l^{-1} for a 22 °Brix must according to Lallemand Inc., Rexdale, Ontario, Canada) within the must is targeted prior to yeast addition for healthy alcoholic fermentation.

Grape growers and winemakers aim for moderate N-status in vines to produce high quality fruit (avoid excessive shoot vigour), and reduce the potential for fermentation haze or conditions that can lead to excess thiol formation (Bell & Henschke, 2005; Beltran, Esteve-Zaraoso, Rozes, Mas, & Guillamon, 2005; Gardner et al., 2002; Vilanova et al., 2007). Few reports exist on specific relationships between individual free amino acids and wine volatiles, but such associations are an area of active research (Hernandez-Orte, Cacho, & Ferreira, 2002; Miller et al., 2007; Ugliano et al., 2009; Vilanova et al., 2007).

Studies have shown that concentrations of N-containing compounds in grapes differ depending upon the analytical method (HPLC condition, derivatisation method, etc.) used to quantify these compounds (Filipe-Ribeiro & Mendes-Faia, 2007; Gump

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et al., 2002). It is also important to understand how different sample preparation methods may affect measurements of nitrogenous compounds in berries. The current sample preparation method used by the winegrape industry prior to making must nutrient supplementation decisions (mainly supplemented with diammonium phosphate, DAP), is simply to crush the grapes and obtain the free run juice fraction (mainly pulp). Though, it has been demonstrated that free amino acids are not distributed uniformly within grape berries and their concentrations vary with the type of grape berry tissue (i.e., skin, pulp, seed) examined (Lamikanra & Kassa, 1999; Stines et al., 2000). Among the current literature, one study (Gump et al., 2002) has contrasted the N-status of 'Cabernet Sauvignon' juices obtained from pressing to those of homogenised grapes, which indicates that preparation steps are often an overlooked item in grape analyses.

The first objective of this study was to contrast free amino acid profiles of grape juice (must) to profiles of whole berries, in order to understand how these sample preparation methods compare to each other. The second objective was to determine whether both methods are equally capable of detecting differences in the N compounds available to yeast (primary free amino acids and ammonia) in fruit produced on vines with varying nutrient regimes.

2. Materials and methods

2.1. Plant material

Grape berry samples (self rooted 'Pinot noir' clone FPS 91, Pommard) were obtained from vines grown in sand-culture (planted in 60 l pots) receiving different levels of either N, phosphorus (P) or potassium (K) supply with other nutrients held constant. The vineyard was planted in 2003 at the Lewis–Brown Research Farm (Oregon State University, Corvallis, OR, USA) in a randomised complete block design with four replicates in each treatment consisting of five continuous vines per replicate.

All vines received complete nutrient solution (half-strength Hoagland's solution; Hoagland & Arnon, 1950) for the first three years after planting (2003–2005), delivered through the drip irrigation system three times per week (Monday, Wednesday, Friday). Vines received water on other days during the growing season (as described below). Three reduced levels of each N, P, or K were supplied to vines in 2006 and 2007. The Control treatment continued to receive a half-strength Hoagland's solution. The concentrations of N, P, and K were each independently supplied at 50%, 20%, or 10% of the concentration used in the Control treatment. A total of ten treatments were applied to 20 vines each (four replicates of five vines) and are designated as; Control, 50% N, 20% N, 10% N, 50% P, 20% P, 10% P, 50% K, 20% K, and 10% K. To clarify, vines in the 50% N treatment received 50% of the concentration of N supplied to vines in the Control treatment, but all other nutrients (including P and K) were supplied at the same concentration as the Control. In this way, only N supply was altered in the low N treatments, only P supply was altered in the low P treatments, and only K was altered in the low K treatments. Vines received ample water throughout the growing season to maintain leaf water potential values above 1.2 MPa. Otherwise, vines were maintained (training, pruning, fungicide applications, etc.) in a similar manner as commercial plantings of 'Pinot noir' grown in the Willamette Valley of Oregon.

All berry samples were collected from the 2007 growing season and all treatments were harvested on the same day. Grapes were picked when a randomly collected composite berry sample reached ~23 °Brix. At harvest, five randomly selected clusters from each replicate were either juiced (details provided later) or frozen immediately. Individual berries were excised while frozen and stored at −80 °C prior to extraction and analysis.

2.2. Reagents, chemicals, and standards

All chemicals for ammonia solution, free amino acid standards, and mobile phases were obtained from Sigma Chemical Co. (St. Louis, MO, USA), unless indicated otherwise. Chemicals for the inline derivatisation prior to HPLC injection were purchased from Agilent Technologies Inc. (Palo Alto, CA, USA). This investigation used analytical and high performance liquid chromatography (HPLC) grade chemicals and solvents.

2.3. Extraction and sample preparation

Half of the randomly selected clusters were juiced, which is the industry standard of sample preparation prior to YAN determination. Stems were manually removed from the clusters and berries were pre-crushed by hand. Crushed berries were pressed in a bench-top scale stainless steel press, and juice (approximately 62% of the berry weight) portion was obtained. Juice samples were frozen at −80 °C until further analyses.

The berries of the other half of randomly selected clusters were fractionated into two portions: skin and pulp, and seeds. Briefly, frozen berries were cut in half and separated into two fractions. Each fraction was placed in liquid N. Excess liquid N was evaporated off and the two fractions were kept at −80 °C until exhaustive extraction.

The skin and pulp fraction was liquid N powdered as described in Lee and Finn (2007), and whole seeds were extracted. These two fractions were combined to make up whole berry extracts. Extraction procedure is described in detail previously (Lee & Finn, 2007; Lee & Martin, 2009), with the following modifications. Water was decided as the extraction solvent for N-containing compounds based on trial extractions of water versus acidified methanol (data not shown). Extraction solvent in this case was high purity water (water obtained from a Millipore Simplicity UV; Millipore Corp., Billerica, MA, USA). Extraction was conducted a total of three times (two re-extractions) and each extract was sonicated for 15 min. The final volume of extracted samples was 25 ml.

All samples were filtered through disposable 25 mm GD/X syringe filters (Whatman Inc., Florhan Park, NJ, USA) prior to ammonia determination and derivatisation.

2.4. HPLC conditions for free amino acids analysis

An Agilent HPLC (HP1100 system; Agilent Technologies Inc.) equipped with a diode array detector (DAD) was used for individual free amino acid analysis. Zorbax Eclipse AAA analytical (4.6 mm × 150 mm, 5 μm, Agilent Technologies Inc.) and guard (4.6 mm × 12.5 mm, 5 μm, Agilent Technologies Inc.) columns were used for free amino acid determination. An autosampler (G1313A; Agilent Technologies Inc.) was used for the inline-derivatisation by *o*-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) immediately prior to injection onto the columns, as described in detail by Henderson, Ricker, Bidlingmeyer, and Woodward (2006). HPLC mobile phase conditions were extended from the gradient programme described by Schuster (1988) and Henderson et al. (2006) to suit our HPLC and column system (Lee, Keller, Rennaker, & Martin, 2009). Briefly, OPA-derivatised amino acids were monitored at 338 nm and FMOC-derivatised amino acids were monitored at 262 nm. Purchased standards of each individual amino acid (Sigma Chemical Co.) were used for identification and quantification (external standard method). Two internal standards, norvaline for OPA-derivatised amino acids and sarcosine for FMOC-derivatised amino acids, were used. Individual free amino acid values were expressed as mg N kg^{-1} of berries fresh weight (mg N kg^{-1} used for conciseness hereafter).

2.5. Ammonia concentration and calculation of YAN values

An enzymatic assay (Sigma ammonia assay kit; Sigma Chemical Co.) using a spectrophotometer (SpectraMax M2 microplate reader; Molecular Devices Corp., Sunnyvale, CA, USA) was used to determine ammonia concentration (NH_3–N). Manufacturer procedures were followed. One-centimetre pathlength disposable semi-micro cuvettes (Fisherbrand, Thermo Fisher Scientific Inc., Waltham, MA, USA) were used.

YAN content was calculated by summing the ammonia values with primary free amino acids as recommended by Filipe-Ribeiro and Mendes-Faia (2007). Primary free amino acid (assimilable amino acid–N; AAA–N) content was determined by excluding HYP and PRO (the two FMOC-derivatised amino acids) content from total free amino acids (FAN) found in berry samples.

2.6. Statistical analysis

Concentrations of individual free amino acids and FAN are expressed throughout this paper in units of mg of N (as opposed to mg of each amino acid). In order to compare N profiles from juiced berries to that from exhaustively extracted berries (so that both measures are expressed in the same units), the juice yield (volume) obtained per unit berry fresh mass was determined. The volume of juice obtained was 0.623 (±0.006SEM) l kg^{-1} berry fresh mass, and was not affected by nutrient treatment ($p = 0.387$) as determined by ANOVA. Violations due to unequal variance between the extracted and juiced samples (that could not be overcome by numerous transformations) excluded our ability to compare both extraction and nutrient treatments in a 2-factor ANOVA. Therefore, we used the Mann–Whitney *U* test (non-parametric test) to compare individual amino acids, NH_3–N, AAA–N and YAN in the juiced versus extracted samples. We examined the effect of nutrient treatments on AAA–N, NH_3–N, and YAN within juice or extracts separately, using a single factor ANOVA. Since AAA–N, NH_3–N, and YAN were not affected by P or K treatments, we further compared individual amino acid profiles in juice and extracts using separate ANOVAs for the N treatments only (Control, 50% N, 20% N, 10% N). Means were compared using Tukey's honestly significant difference (HSD) test at 95% confidence. The impact of nutrient treatments on vine growth parameters, nutrient concentrations in leaves and petioles, and other physiological parameters were analysed using ANOVA. Statistical software (version 8.0, Statsoft Inc., Tulsa, OK, USA) was used for all analysis.

3. Results and discussion

Reduced rates of nutrients supplied to vines in 2006 and 2007 altered the nutrient status of 'Pinot noir' vines, but generally had minimal impacts on vine physiology, growth or fruit yield. Low N supply reduced leaf and petiole N concentrations at bloom and veraison in the 50% N, 20% N and 10% N treatments, but only affected vine growth marginally (shoot length and leaf area of vines measured in spring and summer were unaffected ($p > 0.05$) by N treatments, but dormant season pruning weights were reduced in the 20% N and 10% N treatments; data not shown). Low N supply also reduced K concentrations in petioles at bloom and veraison in the 20% N and 10% N treatments, but increased P concentrations in petioles in 50% N, 20% N, and 10% N treatments. Low P supply reduced petiole P concentrations in 50% P, 20% P and 10% P treatments, but had no impact on growth or pruning weights of vines. Low K supply similarly reduced petiole K concentrations in the 50% K, 20% K, and 10% K treatments without altering growth of vines. Nutrient treatments did not affect leaf gas exchange, chlorophyll fluorescence, leaf water potential or soil moisture in either year. Likewise, nutrient treatments did not alter yield, berry weights, or ripeness (% soluble solids, pH, titratable acidity) of grapes produced in 2007 (will be reported in a forthcoming manuscript).

The whole berry extracts had higher concentrations of AAA–N, NH_3–N, and YAN than the juice samples (Fig. 1), which was mainly due to efficient extraction of the skin N compounds from whole berries (seed FAN contributed <12% of total berry FAN in extracts). AAA–N and YAN had a more than twofold greater concentration in extracts, while NH_3–N was about 33% higher in extracts as compared to juice samples. The nutrient treatments affected AAA–N, NH_3–N, and YAN in a similar manner for both sample preparation methods (Table 1). Only N treatments altered these variables in both juice and extracts. AAA–N was lower in 50% N, 20% N, and 10% N in juices, but only the 20% N and 10% N were lower in extracts. For NH_3–N, only the 20% N and 10% N supply treatments were lower than Control, and this was true in both juices and extracts. YAN was lower in 50% N, 20% N, and 10% N treatments compared to the Control for both juices and extracts. In our analyses, AAA–N was the major contributor to YAN values in both juice and extract, rather than NH_3–N as reported by others (Filipe-Ribeiro & Mendes-Faia, 2007). Some researchers have reported that yeast prefer to utilise N from NH_3 (Arias-Gil et al., 2007; Jiranek, Langridge, & Henschke, 1995), but a combination of AAA–N and NH_3 as a source for N during yeast fermentation may be required for proper wine aroma and flavour development (Bell & Henschke, 2005; Hernandez-Orte et al., 2002). Although, the ratio of AAA–N to NH_3–N is still unknown and may vary with wine style.

With *Saccharomyces cerevisiae* K1 ICV-INRA and a fermentation temperature of 24 °C juice (i.e., must) obtained from 50% N, 20% N, and 10% N treatments would clearly need nutrient supplementation prior to alcohol fermentation due to YAN values below 140 mg N l^{-1} (Bely et al., 1990). If *S. cerevisiae* PYCC 4072 was

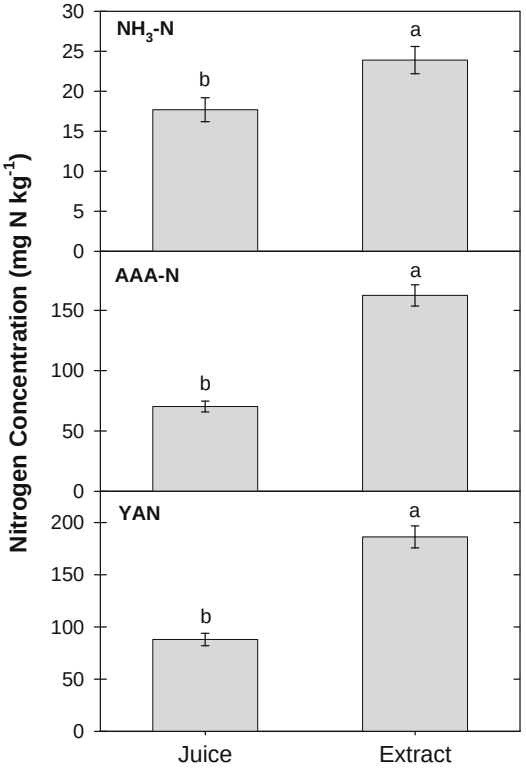

Fig. 1. Effect of sample preparation (juice versus extract) on NH_3–N, AAA–N, and YAN from 'Pinot noir' grapes grown under different levels of N, P, and K supply ($n = 40$). The combined means (SEM) of all nutrient treatments are shown. Analysis from Mann–Whitney test for each variable.

Table 1
Assimilable amino acid–N (AAA–N), ammonia–N (NH_3–N), and total YAN in juice and extracts of 'Pinot noir' grapes grown under different levels of N, P, and K supply ($n = 4$). Values indicate means (standard error of mean; SEM).

Treatment	AAA–N (mg N kg^{-1})		NH_3–N (mg N kg^{-1})		YAN (mg N kg^{-1})	
	Juice[A]	Extract	Juice[A]	Extract	Juice[A]	Extract
Control	92.0 (10) a[B]	193.2 (26) ab	24.8 (7) a	27.8 (6) ab	116.9 (16) a	221.0 (31) ab
50% N	49.9 (10) bc	118.8 (11) bcd	11.9 (3) ab	17.3 (3) abc	61.8 (13) bc	136.1 (14) bcd
20% N	29.5 (3) c	76.5 (7) d	4.9(1) b	7.5 (2) c	34.3 (4) c	84.0 (8) d
10% N	23.5 (3) c	93.8 (11) cd	5.1 (1) b	10.4 (3) bc	28.6 (4) c	104.3 (13) cd
50% P	93.1 (2) a	221.2 (17) a	23.5 (3) a	34.2 (3) a	116.5 (5) a	255.4 (19) a
20% P	92.1 (8) a	221.1 (23) a	19.7 (3) ab	33.0 (5) a	111.8 (11) ab	254.1 (29) a
10% P	75.8 (10) ab	181.8 (17) ab	22.2 (4) ab	28.3 (4) ab	98.1 (14) ab	210.1 (21) ab
50% K	91.5 (9) a	181.0 (16) ab	24.5 (5) a	27.2 (4) ab	115.9 (14) a	208.2 (21) ab
20% K	75.6 (8) ab	168.3 (5) abc	19.0 (4) ab	28.7 (2) ab	94.6 (11) ab	197.0 (6) abc
10% K	80.3 (6) ab	168.2 (15) abc	21.3 (2) ab	24.1 (4) abc	101.7 (7) ab	192.4 (19) abc

[A] Juice values (expressed as mg N kg^{-1}) can be converted to mg N l^{-1} by multiplying by 1.605.
[B] Means followed by the same letter within each column are not different (Tukey's HSD).

planned to be utilised for alcohol fermentation, all juice from this study would need supplementation since a minimum of 267 mg N l^{-1} is needed to ferment to dryness (Mendes-Ferreira et al., 2004). Juice YAN values are an indicator of vine N-status (van Leeuwen et al., 2000) and are used to make N fertilisation decisions for some vineyards in CA (anonymous, personal communication). Based on juice YAN values, the vines from treatments 50% N, 20% N, and 10% N would need fertilisation in the vineyard, but this nutrient study was constructed to determine the minimum required nutrient levels. In future work, it will be interesting to examine if altered P and K status will eventually influence berry amino acids as vines adjust to reduced P and K supply. Bell and Henschke (2005) suspected that variable YAN levels in the past might be due to differences in initial vine nutrient status (vine nutrient reserves). The research samples from this nutrient study will aid in clarifying these questions since vines in this trial received identical nutrient levels for the first three years (i.e., initial vine nutrient reserves were at similar stages) prior to altering nutrient supply.

Twenty-one free amino acids (ASP, GLU, ASN, SER, GLN, HIS, GLY, THR, CIT, ARG, ALA, TYR, VAL, MET, TRP, PHE, ILE, LEU, LYS, HYP, and PRO) were found in both juice and extracts from 'Pinot noir' berries (Tables 2 and 3). Free amino acid composition of 'Pinot noir' berries in this study were similar to Stines et al. (2000), but Stines et al. reported the presence of gamma-amino butyric acid (GABA) and ornithine (ORN) that were not found in our samples. However, Stines et al. (2000) did not detect CIT and TRP in 'Pinot noir' berries as we did based on co-chromatography with purchased standards. CIT was not detected in the seed fraction from 'Pinot noir' berries, which might be due to the dilution introduced during extraction (data not shown).

The extraction method had a significant impact on the relative quantities of amino acids and/or NH_3 (Table 2; compiled values from all nutrient treatments). The relative proportions of 16 out of 21 free amino acids detected were different in juice versus extract. GLU, CIT, TRP, and LYS showed more than a two fold change in relative contribution to total FAN. These same four amino acids, plus ASN, also showed a more than twofold difference in juice versus extract, when expressed as the % of YAN. CIT showed the greatest disparity between extract and juice with a tenfold higher contribution to the juice fraction, but this was also observed in GLU and ALA as proportions of FAN and YAN. These differences might have been due to alterations in FAN contributions from the skin fraction (seeds contributed very little to whole berry FAN; data not shown) compared to free amino acids present in the pulp (mainly what was in juice). The three most abundant sources of free amino acid N were the same in both juices and extracts when examining all free amino acids; HYP > ARG > PRO, though ARG was one of the few amino acids that was not affected by extraction method. The relative proportion of NH_3–N was also different between juices and extracts, again due to the additional skin and seed contributions of NH_3–N in whole berry extracts.

The impact of lowering N supply to vines on free amino acid profiles and content are summarised in Table 3. Seventeen of the 21 amino acids were reduced by low N supply in juice. Only GLU, GLN, TRP, and HYP were not affected by N supply in the juice samples. The total FAN concentration in juice was reduced by more than 100% (twofold) in the 20% N and in the 10% N supply treatments. N supply had an overall lower impact on individual free amino acid concentrations in the extracts compared to juice results. Only 10 of 21 amino acids were significantly reduced by low N supply to vines. In addition, the two lowest N supply rates reduced the total FAN concentration in extracts by about 50%. Interestingly, berries from the lowest N supply vines were not different from the Control in total FAN concentrations in the extracts; furthermore, only the 20% N supply treatment was in that regard.

Table 2
Relative concentration of individual free amino acids and ammonia in juice versus extracts of 'Pinot noir' grapes grown under different levels of N, P, and K supply. These proportions are based on data expressed as mg N contributed from each amino acid or NH_3. The combined means (SEM) of all nutrient treatments are shown ($n = 40$).

N-source		% of free amino acid–N		% of YAN	
		Juice	Extract	Juice	Extract
Aspartic acid	ASP	2.0	1.5	2.9	2.7
Glutamic acid	GLU	6.6 a[A]	2.1 b	9.3 *a*	3.6 *b*
Asparagine	ASN	0.5 b	0.8 a	0.7 *b*	1.4 *a*
Serine	SER	2.9 a	2.2 b	4.1	3.7
Glutamine	GLN	6.1	5.4	8.6	9.6
Histidine	HIS	1.4 b	1.6 a	1.9 *b*	2.6 *a*
Glycine	GLY	0.2 a	0.2 b	0.3	0.4
Threonine	THR	2.3 b	2.8 a	3.2 *b*	4.7 a
Citrulline	CIT	0.5 a	0.1 b	0.7 *a*	0.1 *b*
Arginine	ARG	25.7	26.3	33.7 *b*	41.4 *a*
Alanine	ALA	5.4 a	4.0 b	7.3 *a*	6.6 *b*
Tyrosine	TYR	0.2 b	0.2 a	0.2 *b*	0.4 *a*
Valine	VAL	1.6 a	1.4 b	2.3	2.4
Methionine	MET	0.3 b	0.4 a	0.3 *b*	0.6 *a*
Tryptophan	TRP	0.6 b	1.2 a	0.8 *b*	2.1 *a*
Phenylalanine	PHE	0.6	0.5	0.8	0.9
Isoleucine	ILE	1.1 a	0.9 b	1.6	1.6
Leucine	LEU	1.3	1.2	1.7 *b*	2.0 *a*
Lysine	LYS	0.3 b	0.6 a	0.4 *b*	1.1 *a*
Hydroxyproline	HYP	26.7 b	35.1 a	–	–
Proline	PRO	13.7 a	11.4 b	–	–
Ammonia	NH_3	–	–	19.1 *a*	12.2 *b*

[A] Letters designate significant difference between juice and extract within each variable based on Mann–Whitney test; '–' stands for not applicable.

Table 3
Effect of N supply on individual free amino acid concentrations in juice and extracts of 'Pinot noir' grapes ($n = 4$).

N-source	Juice (mg N kg^{-1})				Extract (mg N kg^{-1})			
	Control	50% N	20% N	10% N	Control	50% N	20% N	10% N
ASP	2.3 a[A]	1.9 ab	2.0 ab	1.7 b	5.1	4.3	3.9	4.1
GLU	7.4	6.2	5.3	4.6	6.4	5.2	4.2	5.9
ASN	0.6 a	0.4 ab	0.4 ab	0.3 b	2.3	2.1	2.0	2.1
SER	3.8 a	2.7 b	1.9 bc	1.6 c	7.1 *a*	5.6 *b*	3.8 *c*	4.6 *bc*
GLN	6.7	5.5	4.9	3.9	15.1	13.4	13.2	17.2
HIS	2.3 a	1.2 b	0.7 b	0.5 b	5.3 *a*	3.5 *b*	2.7 *b*	2.9 *b*
GLY	0.4 a	0.2 b	0.1 b	0.1 b	0.7	0.9	0.4	0.4
THR	3.5 a	2.0 b	1.2 bc	0.9 c	10.3 *a*	6.3 *b*	3.9 *b*	4.6 *b*
CIT	0.9 a	0.4 b	0.2 b	0.2 b	0.2 *a*	0.1 *ab*	0.1 *ab*	0.1 *b*
ARG	48.5 a	19.9 b	6.5 b	4.4 b	107.3 *a*	53.1 *ab*	23.3 *b*	29.1 *b*
ALA	8.6 a	4.5 b	2.5 b	2.0 b	13.7 *a*	8.8 *ab*	5.7 *b*	6.8 *b*
TYR	0.2 a	0.1 b	0.1 b	0.1 b	0.7	0.6	0.5	0.6
VAL	2.1 a	1.5 ab	1.0 b	0.9 b	4.7 *a*	3.3 *b*	2.6 *b*	3.4 *b*
MET	0.3 a	0.2 ab	0.1 b	0.1 b	1.0	0.8	0.7	0.8
TRP	0.6	0.5	0.4	0.4	3.4	3.1	2.9	3.2
PHE	0.6 a	0.5 ab	0.3 ab	0.3 b	1.4	1.1	1.0	1.2
ILE	1.3 a	1.0 ab	0.8 ab	0.7 b	2.9 *a*	2.2 *ab*	1.9 *b*	2.5 *ab*
LEU	1.6 a	1.1 ab	0.7 b	0.6 b	3.8 *a*	2.8 *b*	2.2 *b*	3.0 *ab*
LYS	0.3 a	0.2 ab	0.2 ab	0.2 b	1.9	1.7	1.6	1.5
HYP	26.6	22.4	23.5	21.3	79.1	92.0	101.7	105.5
PRO	20.8 a	16.3 ab	5.3 b	4.9 b	40.0 *a*	32.3 *ab*	16.6 *c*	22.6 *bc*
Total FAN	139.4 a	88.6 ab	58.3 b	49.7 b	312.4 *a*	243.2 *ab*	194.8 *b*	221.9 *ab*

[A] Letters indicate significant differences (Tukey's HSD) between N treatments within each of the juice or berry extract values.

In general, the 10% N supply treatment had the lowest concentrations of free amino acids in juice, but the 20% N supply treatments were most often the lowest in extracts. This lack of distinction between juice and extracts might be due to the greater impact on ARG (based on concentration) by altered N supply, which was the main free amino acid reported in the 'Pinot noir' juices (pulp fraction; Huang & Ough, 1989, 1991). If these grapes were to be processed into red wine, exhaustive extraction would give a more complete picture of their true N-status. If these grapes were to be fermented into white wine, YAN values obtained from juice would likely be a better indicator of N-status.

In general, the majority of the free amino acids (19 out of 21; Table 3) were lower in juice samples compared to extracts, except for GLU and CIT levels which were higher in juice samples. It is possible that these two amino acids could have degraded during the more extensive process of exhaustive berry extraction.

Berry size (i.e., skin to pulp ratio) and other indirect growth effects did not impact the findings in this study. Other studies have noted the importance of comparing free amino acid composition of grapes at similar % soluble solid content (Gump et al., 2002; Kliewer, 1971; Stines et al., 2000). The differences in free amino acid levels and profiles exhibited in this study were due to nutrient effects, and not due to variations in harvest % soluble solids (i.e., differences in maturity) since treatments did not alter °Brix.

ARG was the major free amino acid in Control juice, followed by HYP then PRO, which was the same trend observed in Control extracts (Table 3). The major free amino acid in the juices varied with lower N treatments (HYP was predominant in 50% N, 10% N), but remained ARG in the low P and K treatments (data not shown). Our results indicate 'Pinot noir' grapes from N deficient vineyards will not have the typical free amino acid profile as observed in N deficient 'Cabernet Sauvignon' and 'Thompson seedless' grapes of past studies (Kliewer, 1971; Kliewer, Bogdanoff, & Benz, 1991; Rodriguez-Lovelle & Gaudillere, 2002).

Rodriguez-Lovelle and Gaudillere (2002) reported a significant quantitative difference in ARG, GLN, ALA, and HIS in their 'Cabernet Sauvignon' grapes from N deficient vines, with ARG exhibiting the most drastic difference. This distinction may have been due to ARG's role as the major storage pool in grape berries (Kliewer, 1971; Rodriguez-Lovelle & Gaudillere, 2002). Bath, Bell, and Lloyd (1991) reported a positive linear trend between vine N applied and ARG levels in 'Sauvignon blanc' juice during the second year of their study, but not from the first year, indicating that initial vine nutrient level was important in interpreting these results. Rodriguez-Lovelle and Gaudillere (2002) speculated that ARG was the indicator compound most related to vine N-status, which was also demonstrated in 'Thompson seedless' grapes by Kliewer (1971).

Huang and Ough (1991) reported ARG and PRO to be the major free amino acids in 'Pinot noir' juice samples; they found 289 mg N l^{-1} of ARG and 36 mg N l^{-1} of PRO in 45 samples from CA during the 1988 growing season. Huang and Ough (1989) found ARG levels of 29.9 mg N l^{-1} and PRO levels of 9.7 mg N l^{-1} in 'Pinot noir' juice samples from Oakville, CA. Stines et al. (2000) reported 339 mg N kg^{-1} of ARG and 154 mg N kg^{-1} of PRO in their chemically extracted 'Pinot noir' whole berries (entire berries were liquid N powdered then extracted with a mixture of methanol, chloroform, and water). Spayd and Andersen-Bagge (1996) reported PRO > ARG in 'Pinot noir' juices from samples collected in 1987 and 1988, then PRO < ARG for seasons 1989 and 1990 (from values expressed as mg N l^{-1}), demonstrating free amino acid variation due to growing season.

A linear relationship between juice YAN and extract YAN accounted for nearly 80% of the variation between the two sample preparation methods examined here (Fig. 2). This relationship can be used to approximate juice YAN concentrations (a value familiar to winemakers; expressed in mg N l^{-1}) from whole berry extraction data, which are becoming more common in research (Lamikanra & Kassa, 1999; Stines et al., 2000). Actual YAN required by yeast during fermentation is probably flanked by values obtained from juice and those of exhaustively extracted samples, since the initial fermentation of red winemaking occurs with skin and seed contact, as suggested by Stines et al. (2000). Variation in skin to pulp ratio, skin and seed contact time, etc. will alter the YAN levels available during red winemaking. Overall higher levels of YAN from extracts compared to juices were mainly from the efficient extraction of YAN present in the skin, since the seed fraction contributed only a small amount (seed YAN was <4% of whole berry YAN).

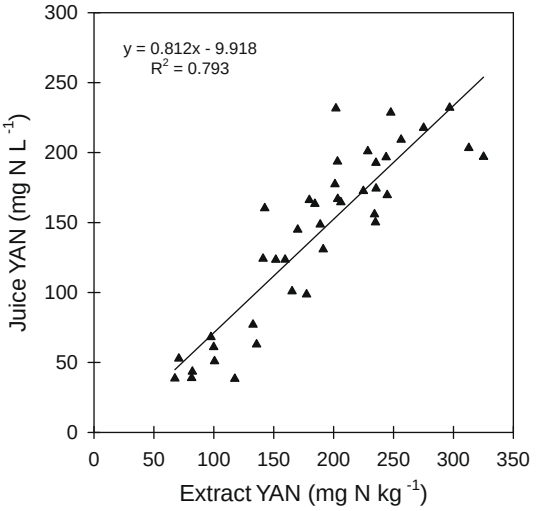

Fig. 2. Relationship between juice YAN and extract YAN values obtained from different subsamples of 'Pinot noir' grapes in 2007.

4. Conclusions

The findings in this study emphasise the importance in unifying the extraction procedure when free amino acid profiles of grapes are determined. Caution should be given when comparing values of YAN in the literature utilising different sample preparation methods.

Future work should be conducted to examine the YAN levels in grape and winemaking fractions obtained before and after fermentation (must, whole berry, and presscake) to gain some insight into how much YAN actually is depleted from separate grape fractions. We suspect the value will fall between those of juiced and exhaustively extracted YAN preparations.

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