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Optimization of an array of Peptidic Indicator Displacement Assays for the Discrimination of Cabernet Sauvignon Wines

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Optimization of an array of Peptidic Indicator Displacement Assays for the Discrimination of Cabernet Sauvignon Wines

by

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Report

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Dedication

To my family: my gracious and wonderful mom, my beloved - Travis, my nieces – Samantha, Stephanie, and Savannah, my sisters – Susie and Sandie and my brothers in law – Eddie and Ralph. Blessed be.

Abstract

Optimization of an Array of Peptidic Indicator Displacement Assays for the Discrimination of Cabernet Sauvignon Wines

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The research project, *Optimization of an array of Peptidic Indicator Displacement Assays for the Discrimination of Cabernet Sauvignon Wines*, describes the multiple step lab trials conducted to optimize an array of ensembles composed of synthesized peptides and PCV:Cu^{+2} complexes for the differentiation of seven Cabernet Sauvignon wines with different tannin levels. This report also includes the methods and analysis used. The analysis interpreted by principal component analysis**.**

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CHAPTER 1: INTRODUCTION

The overall goal is to construct an array of indicator displacement assays that can fingerprint for complex mixtures. Current work being done is to improve methods for environmental testing, online process monitoring, proteomics – a large scale study of proteins, metabolomics – study of the unique chemical fingerprints that specific cellular processes leave behind, medical diagnosis such as disease status, and risk markers. The short term goal for this research report is expanding the loading plot (PCA) from current work done, assisting to discover a peptide that helps with the discrimination between not just different wines, but wines that are the made from the same kind of grapes.

1.1 PURPOSE OF STUDY

The purpose of this study was to improve the array of peptic receptors using an indicator displacement assay (IDA) to discriminate between seven Cabernet Sauvignon wines. The seven wines were selected from a collection of 17 different wines which differ in tannin levels. Principal component analysis - PCA was the method of choice for statistically and visually interrupting the results obtained.

1.2 RESEARCH OBJECTIVES

This study was an attempt at optimizing an array of peptidic sensors that could discriminate complex mixtures. Cabernet Sauvignon wines are used as test complex mixtures. In this study, specific objectives were:

1. To differentiate Cabernet Sauvignon wines which have been harvested at different stages of maturation.

2. To determine the peptide sequence that differentiates the Cabernet Sauvignon wines.
3. To determine whether differentiation of the Cabernet Sauvignon wines is correlated with their tannin level.

1.3 SCOPE OF THE RESEARCH

This research study was subdivided into four phases. The first phase was conducting a literature review of information pertinent to this study.

The second phase of the study was developing or revisiting concepts or skills needed to perform the synthesis of the peptides, purification process or conducting of the experiments. The concepts included were Beer's Law, molarity and dilution, milli-molar and micro-liters concepts, UV-vis spectrophotometry (Beckman Coulter DU® Spectrophotometer and Molecular Devices SpectraMax® Plus^{384} - microplate spectrophotometer), evaporation (Buchi Rotavapor® R11), automated solid phase peptide synthesis (the Protein Technologies, Inc. Prelude ™), freeze drying (LABCONCO Lyophilizer), solid phase extraction (SPE), and high performance liquid chromatography (HPLC).

The third phase of the study was to synthesize and purify tripeptides, pentapeptides and polypeptides. These will serve as receptors in sensing ensembles which function via indicator displacement. The sensing ensemble is composed of peptides, metal ions, and pH indicators. It was previously found that an array of indicator displacement assays could discriminate various varietals of wines (Umali, A. et al., unpublished results). The same array of sensing ensembles was tested for its ability to discriminate Cabernet Sauvignon wines harvested at different stages of maturation. However results were not satisfactory.

Theses results prompted the fourth phase of the study: the analysis of Cabernet Sauvignon wines obtained from UC Davis. The UC Davis project involves determining the relation of the wine sensory attributes and the time of wines' harvest. It is hoped that the optimized array of peptidic sensing ensemble can effectively discriminate Cabernet Sauvignon wines harvested at different times.

CHAPTER 2: BACKGROUND

2.1 SUPRAMOLECULAR ANALYTICAL CHEMISTRY

Supramolecular analytical chemistry is "a relatively new cross-disciplinary field of chemistry…. It encompasses all of the disciplines and subdisciplines involving intermolecular interactions… and primarily associated with chemists working on organic and/or inorganic synthetic structure" (Anslyn, 2007, p.687). These structures being synthesized are sensors.

A sensor is a mechanical device sensitive to light, temperature, radiation level, or the like, that transmits a signal to a measuring or control instrument as defined by The American Heritage Dictionary. No mechanical device or control instrument is utilized; therefore the sensor is as stated by Anslyn (2007), a receptor that interacts with the analyte causing evidential change much like an indicator. In concordance Zhang & Suslick (2005) also state that a pH indicator was not just indicative of the change in pH values. In their study, they maintained all analyte solutions at a pH value of 7.0 to then attribute the color change indicative of the dye interaction with the analyte, not due to a change in pH values. The color changes as reported by Zhang & Suslick were as unique as fingerprints for each specific analyte mixture.

2.2: INDICATOR DISPLACEMENT ASSAY

The method known as Indicator Displacement Assay encompasses the noncovalently bonded indicator (pryocatechol violet - PCV) to a host (copper^{2+}) and an addition of an analyte (wine). Unbound indicators and hosts have their own characteristic color but as soon as the indicator (PCV) is noncovalently bonded to the host (Cu^{2+}) a color change is observed. Once the analyte and the indicator are both in the reaction

vessel, the competition for the bind site begins. When the host binds with the analyte it of the analyte, the structures of the indicator and a component in the analyte must be similar and in competition for the host, which once again includes a visual observation of a color change (Wiskur & Anslyn (2001). As stated in an article by Zhong and Anslyn 2002, indicator displacement assays are used to convert synthetic receptors into sensors. An 'optical' visual signal is observed to be indicative of when an analyte has bound to the host by means of displacing an indicator species.

As explained in a study by Collins & Anslyn (2007), the receptor is first introduced to the indicator that binds in the receptor binding site, and then the analyte displaces the indicator. Hence the analyte and the indicator are in competition for the binding site. A useful dynamic part of IDA to keep in mind is the solvent for which IDA utilizes. As described by Anslyn (2007), the binding of the analyte to the receptor is optimal near the dissociation constant (K_d), "the basis of an IDA is a protonation change of the indicator when bound versus free in solution" (p. 691).

As stated by Shabbir, Regan, & Anslyn (2009):

> An indicator displacement assay relies on a colorimetric or fluorescent indicator that changes optical or electrochemical properties when bound to a host relative to being free in the bulk medium. The most commonly used indicators are pH indicators. The competition between an indicator and the guest of interest for the binding site of the host allows the determination of total guest (or analyte) concentration. An IDA both eliminates the need to incorporate the chromophore or fluorophore into the structure of the host, thus simplifying the synthesis of the host molecule, and allows one to tune the sensitivity of the assay because of the ability to change the identity and concentration of the indicator.

According to chapter six of Titrimetric Analysis, the most typical complexometric indicators are metallochromic indicators. Due to the reversible complex formation reactions usually cause a color change or two. Metal complexing Indicator Displacement Assay utilizes partially filled 3d transition metals. It is the transition metals partially filled orbitals' that allow it to act as "a chromophore or fluorophore quencher" in particular Copper $^{2+}$. As per Nguyen & Anslyn (2006), it requires two copper ions to gain affinity for the analyte resulting in higher selectivity.

2.3 PATTERN RECOGNITION – PRINCIPAL COMPONENT ANALYSIS

In a study by Kitamura, Shagufta, & Anslyn, 2009, the analysis of complex mixtures and diverse chemical structures is made possible by pattern recognition. Pattern recognition takes advantage of the arrays' responses as a collective whole, not which receptor responded to which analyte.

The fact that the synthetic receptor does not have to bind selectively or with high affinity to a target analyte makes pattern recognition a commanding tool. This is because as long as the receptor binds differentially to the analytes and creates different patterns the basics of pattern recognition have been achieved. Due to the large amounts of data, a multivariate analysis such as principal component analysis (–PCA) is necessary. PCA forms the basis for a multivariate data analysis with N# rows of observations and M# of columns of Variables. According to Eriksson et al. (2006) when the data is analyzed from a loading plot the further away from the origin a variable lies the stronger the impact that variable has on the model.

CHAPTER 3 EXPERIMENTAL METHODOLOGY

3.1 PREPARATORY METHODS

Before the actual testing on Cabernet Sauvignon wines, all the necessary preparatory precursors had to be synthesized, purified, and familiarization on instrumentation had to be completed.

The first activity was the familiarization with the Molecular Devices SpectraMax® Plus^{384} Spectrophotometer utilized in order to find the binding curve of a peptide sensor for the metal complexing Indicator Displacement Assay of quercetin, a tannin found in wine: Incorporated steps involved were: making a 500.0 ml of a 50mM solution of HEPES in a 1:1 of ethanol:water (etOH:H_2O) at a pH of 7.4 by the use of a pH meter and varied concentrations of sodium hydroxide and hydrochloric acid. By the utilization of the Molecular Devices SpectraMax® Plus^{384} Spectrophotometer shown in Figure 3.1, the [Cu^{2+}]:[PCV] – Pyrocatechol violet indicator binding curve was determined to be a 1:1 ratio as shown in Figure. 3.2 (a). With the knowledge obtained about the binding curve of copper II and PCV, a well plate trial was run with 0.75 mM PCV, 0.75 mM of Copper solvated from copper (II) sulfate pentahydride, and a 0.75mM of quercertin at different volumes of quercetin for a combined volume of 300 ul. The stock solutions were calculated and prepared to be 0.75mM. The result of [PCV]:[Cu^{2+}]:[Qt] - quercertin binding ration was determined to be a 1:1 as depicted below Figure 3.2 (b). These results were obtained using the Beckman Coulter DU® 800 Spectrophotometer.

Figure: 3.1 Molecular Device SpectraMax®

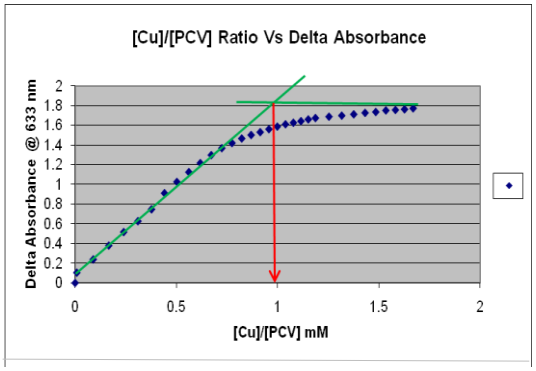


Figure: 3.2 (a) [Cu2+]:[PCV]

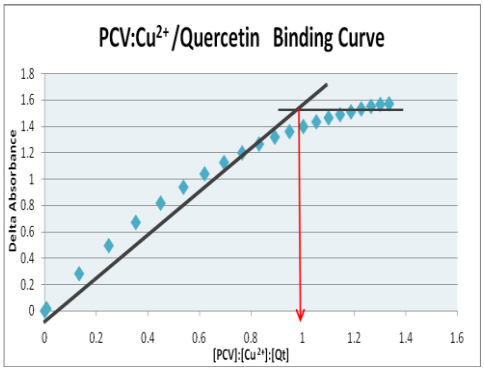


Figure: 3.2 (b) [PCV]:[Cu2+]:[Qt]

3.2 PEPTIDE SELECTION AND SYNTHESIS

The amino acid selections that created the peptide were selected due to previous findings. In the study by Wright, Anslyn, & McDevitt (2005), it was concluded that the peptide arms are important for discriminating between the individual tripeptides. The tripeptide consisting or His-Gly-Thr had the greatest interaction compared to other tripeptidic arms. If a peptidic arm had sufficient discrimination for that study, perhaps a

sensing ensemble containing histidine and glycine have a definitive interaction for this study. The syntheses of the peptides were composed as shown in the table 1 below:

Table: 1. Peptide sequences

	Peptide Name	**Peptide Sequence**
1	SC1	Met-Trp-Gly (MWG)
2	SC2	His-Trp-Gly (HWG)
3	SC3	Asn-Val-Met-Trp-Gly (NVMWG)
4	SC4	Asn-Val-His-Trp-Gly (NVHWG)
5	LT1	Trp-Asp-Asp-Cys-Asp-Asp (WDDCDD)

The synthesis of the polypeptides was a multistep process. Solid Phase Peptide Synthesis-(SPPS) is the method used to synthesis the above mention polypeptides. Umali, A. explained the two terminus functional groups on an amino acid could either be carboxyl group or the amino group because they can interact with each other, when the peptides are synthesized a protected pre-loaded resins must be used to keep the amino group from reacting to other undesirable groups (personal communication, June 28, 2010). The pre-loaded resins used were Wang resins. Fmoc chemistry (fluorenylmethyloxycarbonyl) were utilized. That required the C-terminus to act as the starting point, hence synthesis starts on the right hand side working backwards, meaning that SC1-Met-Trp-Gly was constructed in the order of Gly-Trp-Met.

The automated SPPS Protein Technologies, Inc. Prelude Model was used as seen below in Figure 3.3. The first step was making the solutions and weighing out the amino acids needed as shown on Table 2 and Table 3. The steps are as followed for the

synthesis of the peptide preparation: The resin amounts were weighed out in reaction vessels (RV) and placed in holders. The Fmoc amino acids were dissolved in DMF and then transferred into designated amino acid bottles and covered with parafilm. The solvents were made and placed in designated glass bottles. The Prelude instrument was prepared and programmed. The RVs and preloaded resins were placed onto the Prelude, and the program was started.

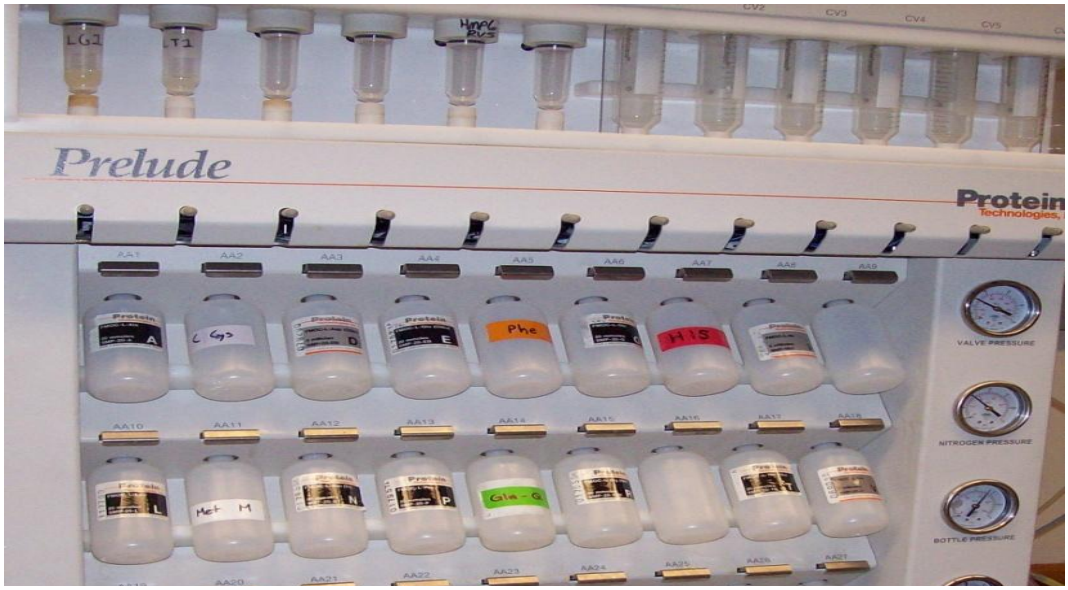


Figure: 3.3 Protein Technologies Inc. Prelude with loaded RVs.

Table 2: Solutions for peptide synthesis

Name of solution	Volume (ml)
Dep 20% Piperidine/DMF	200 ml
PyBOP 300 mM PyBOP	100 ml
LiCl 0.8 M LiCl/DMF	150 ml
DIPEA 1.2 M DIPEA	50 ml

Table 3: Amino Acid weights

	Abbr.	Amino Acid	Weight (mg)	Volume (ml)
1	C	Cysteine	1523 mg	13 ml
2	D	Aspartic Acid	1564 mg	19 ml
3	G	Glycine	1308 mg	22 ml
4	H	Histidine	2355 mg	19 ml
5	M	Methionine	1189 mg	16 ml
6	N	Asparagine	1909 mg	16 ml
7	P	Proline	877 mg	13 ml
8	Q	Glutamine	1588 mg	13 ml
9	V	Valine	1086 mg	16 ml
10	W	Tryptophan	2949 mg	28 ml

This process normally takes between one to two days; it took three days due to a hose not being submerged into its solvent. The instrument's last process was to couple the last Fmoc amino acid. Then the 'Deprotection' step was started; this step removes the last Fmoc group from the amino acid chain. One of the washing processes began. The RVs were removed from the Prelude and placed in a rack for transportation into the other lab area.

The peptides were then prepared for the cleavage of resin. This process described below as it took place under the fume hood. A filtration system was setup by obtaining a 125 ml filter flask fitted with a septum stopper. A syringe needle was inserted in to the stopper; the RV sits onto the top of the syringe needle. A hose was attached to the side arm of the filter flask. The peptide was washed with 5 ml glacial acetic acid three times

for a total of 15 ml of glacial acetic acid. As the glacial acetic acid was washing the peptide the RV was slightly agitated for a thorough washing. Caution the RV sits on the syringe needle loosely see Figure 3.4(a). This step was repeated two more times; once with DCM (same procedure of 5 ml three times), and one last time with 15 ml total of methanol. A parafilm cover was placed on the RVs and tiny holes were made with a syringe see Figure 3.4(b). The RVs were placed into the desiccator and left over night.

Figure: 3.4(a) RV in syringe needle

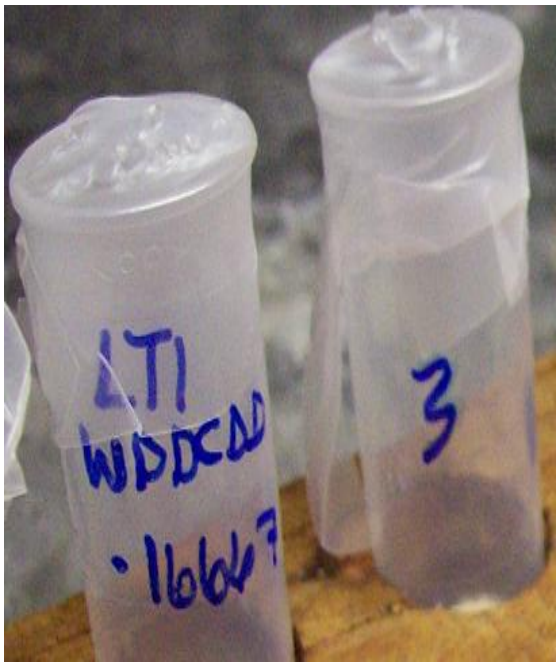


Figure: 3.4 (b) Parafilm cover with holes.

Two cleavage solutions were prepared to remove the resin bead from the peptides. Theses steps took place under the fume hood. Two different kinds of solutions were needed to accommodate for the peptides having Cys or Met, they required a solution of 94% TFA-Trifluoroacetic acid, 2.5% water, 2.5% EDT-Ethanedithiol, and 1% TIS-Triisopropylsilane, while those peptides not containing Cys or Met took a cleavage solution of 95%TFA, 2.5 % water, and 2.5% TIS. A plastic cap was placed under each

RV, then 5ml of cleavage solution was pipetted into each RV, parafilmed and gentle swirled. With occasional swirling, the RVs needed to stand for at least four hours.

For the ether precipitation a filtration setup was set up. The setup was as follows: syringe needle, septum stopper, suction adapter, 100 ml round bottom flask, clamp, and a hose. The plastic cap from the RV was removed and placed on top of the syringe needle. The vacuum set up was checked, then the washing of the resin took place. The first was consisted of three - 5 ml volumes for a total of 15 ml with TFA. The TFA was evaporated using the Buchi Rotavapor® R11 see Figure 3.5(a). An ice bath was prepared where 50 ml of diethyl ether was placed in a 150 ml Erlenmeyer flask and cooled while the evaporation took place. Once the TFA was evaporated the residue was cooled see Figure 3.5 (b). Cold diethyl ether is added to the cold flask, and the peptide precipitated see Figure 3.5 (c). The peptide is washed with ether and decanted this was done twice. A small sample was obtained to run a HPLC chromatograph. The remained of the peptide was transferred into a centrifuge tube by dissolving the peptide in minimum amount of deionized water. Centrifuge tubes should not be more than half filled.

Figure: 3.5(a) Rotavap

Figure: 3.5(b) cooling residue

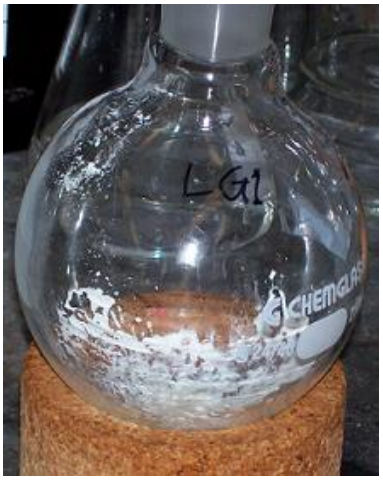


Figure: 3.5(c) Peptide residue

The lyophilization of peptide samples was next. A dry ice acetone bath was prepared in a doer. Centrifuge tubes were immersed into bath for approximately 30 minutes or until peptide sample was frozen see Figure 3.6(a). Once peptide samples were frozen the blue caps were labeled and removed and a Kimwipe® was folded in half and placed around the mouth of the centrifuge tube. A rubber band was used to keep the Kimwipe® in place. Centrifuge tubes were taken to the Freeze Dryer to be Lyopholized see Figure 3.6(b). After 24 to 48 hours, samples were removed and recapped their appropriate caps. Samples were taken back to the lab.

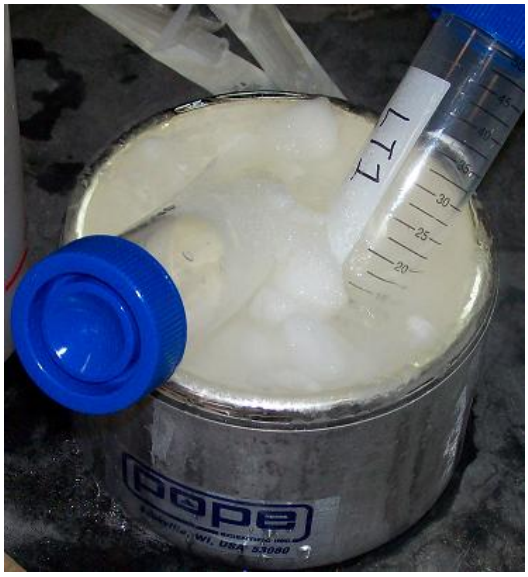


Figure: 3.6(a) Dry ice –acetone bath

Figure: 3.6(b) Freeze Dryer

3.3 PEPTIDE ANALYSIS AND PURIFICATION

Peptide samples extracted before the lypolization process began were then prepared to be run through a High Performance Liquid Chromatography –HPLC see Figure 3.7(a). The process began with cleaning and priming the HPLC, solvents were made 0.1% TFA in distilled deionized water and 0.1% TFA in acetonitrile. After

solutions were made they had to be filtered before they could be run on the HPLC see Figure 3.7(b).

Figure: 3.7(a) Beckman Coulter Sys. HPLC & Close up

Figure: 3.7(b) Filtration

Samples were then prepared, by dissolving in minimum amount of deionized water. The sample was then filtered through a filter syringe and plastic syringe. The filtered solution was then squirted into a small vial and labeled appropriately see Figure 3.8 HPLC samples were run and chromatographs were analyzed. Below HPLC Chromatographs are shown see Figure 3.9(a) – LG1, Figure 3.9(b) – LT1 Figure 3.10(a) - SC1, Figure 3.10(b) - SC2, Figure 3.11(a) - SC3, and Figure 3.11(b) – SC4. The circles around the peaks are the desired product, if not other peaks are seen on chromatograph then the peptide sequence was pure. LG1, LT1, SC1, and SC2 had to be purified.

Figure: 3.8 Labeled vials filled with peptide samples awaiting HPLC test.

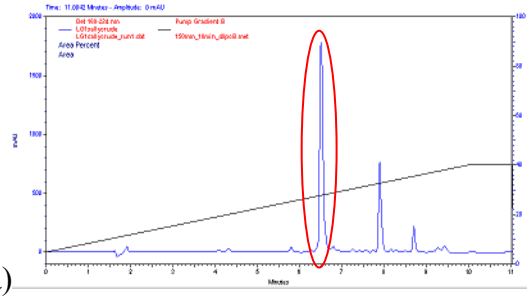
(a) 

Figure: 3.9(a) Chromatograph of LG1

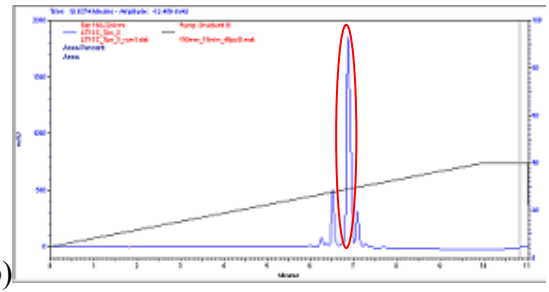
(b) 

Figure: 3.9(b) Chromatograph of LT1

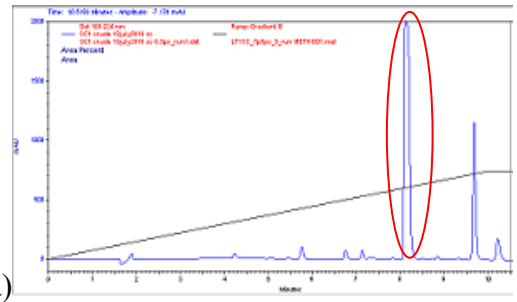
(a) 

Figure: 3.10(a) Chromatograph of SC1

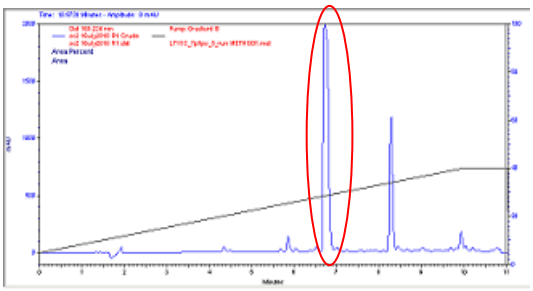
(b) 

Figure: 3.10(b) Chromatograph of SC2

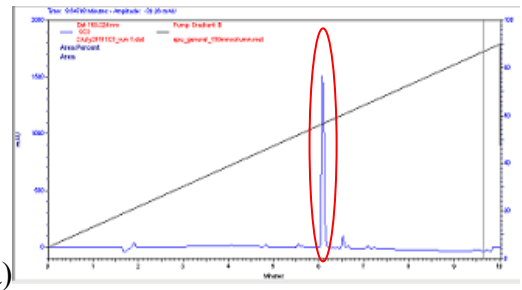
(a) 

Figure: 3.11(a) Chromatograph of SC3

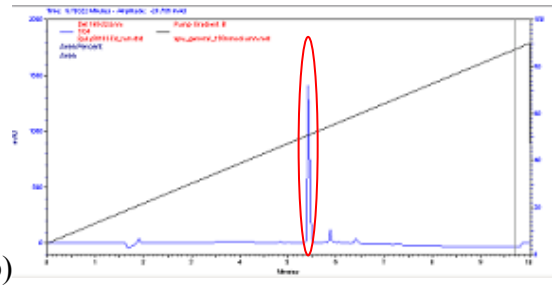
(b) 

Figure: 3.11(b) Chromatograph of SC4

By looking at the HPLC chromatographs, the following peptides needed to be purified: LG1, LT1, SC1, and SC2. The purification process utilized was Solid Phase Extraction. The crude impure peptide was diluted with 1 ml of 0.1% TFA –H_2O. Meanwhile, two solutions were prepared 100 ml of 0.1 % TFA in distilled deionized water, and 100 ml of 0.1% TFA in acetonitrile. The solutions were used to make

different gradients of 0.1% TFA-ACN in 0.1% TFA-H_2O. The gradients began with 5%, 7%, 10%, 15%, 20% of ACN in H_2O. A Waters Sep-Pak Vac 20 CC, C18 column was utilized. A small stopcock and hose were attached to the Sep-Pak. The Sep-Pak had to be equilibrated by letting 10 ml of 0.1%TFA ACN run through the column, then 10 ml of 0.1% TFA –H_2O. Before the column was dry, the stopcock was closed. The dissolved impure peptide was pipetted into the Sep-Pak, the sample was delivered a small amount at a time to avoid getting the rest of the peptide all over the column see Figure 3.12 (a). The smallest surface area of the column in contact with the peptide the better, this allowed the peptide to seep through the column of C18 to noncovalently attach to the particles in column. When different concentrations of TFA/ACN were run through the column the eluted parts were collected in to a syringe see Figure 3.12(b). The elution was then filtered through a syringe filter. The squirted filtered solution was inserted into a small vial to await an HPLC test. When a clear desired peak was observed then the column was flushed with this concentration to ensure that only this portion eluted out. Then a gradual concentration of 0.1% TFA ACN additive to the known percentage was used until the desired peak was not longer observed in the HPLC chromatograph. LG1 was not purified due to time constraints.

Figure: 3.12(a) SPE Figure: 3.12(b) SPE setup

3.4 BINDING RATIOS

Pyrocatechol violet is an indicator that has two catechol or hydroxyl functional groups see illustration 1 (a). Tannin or flavonoids contain catechol functional groups as well. This is imperative because there must be a level of hydroxyl/catechol competition between the indicator and the analyte. In this study the indicator is Pyrocatechol violet – PCV see illustration 1 (b), and the metal complexone is copper II, which attaches to the PCV. When the analyte, wine is introduced to the reaction well copper becomes bound to the tannin leaving the PCV unattached. A visual indicator is observed, a color change takes place see illustration 2.

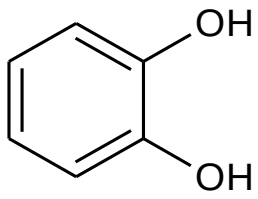


Illustration: 1(a) – Catechol structure

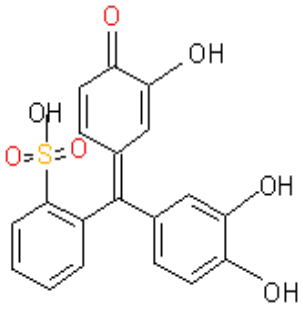


Illustration: 1(b) – PCV structure

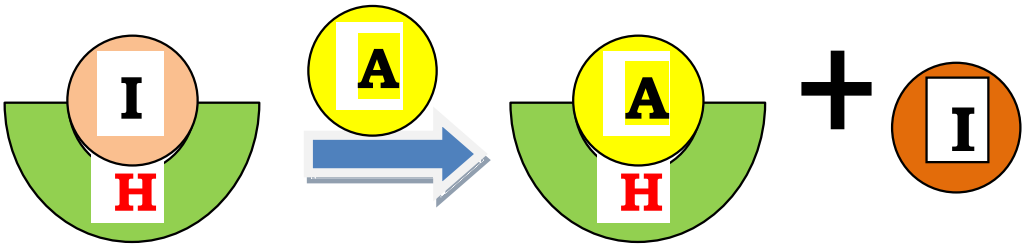


Illustration: 2 – I -Indicator = PCV, H - Host = copper, and A – Analyte = Wine/Tannins

The binding curves for the peptides were determined by Pyrocatechol violet, copper II, and the addition of a peptide at different amounts. All stock solutions were 0.75mM of PCV, Cu^{2+}, Peptides, 50mM HEPES in 1:1 EtOH:H_2O at a pH of 7.4. A 96 well plate was prepared with no more than 300 ul of solution per well. 50 ul of both PCV and Cu^{2+} were utilized. The amount of peptide varied as did the volume of the HEPES. The Molecular Devices SpectraMax® Plus^{384} Spectrophotometer was utilized. For the binding curves see Figures 3.13(a) and Figure 3.13(b)

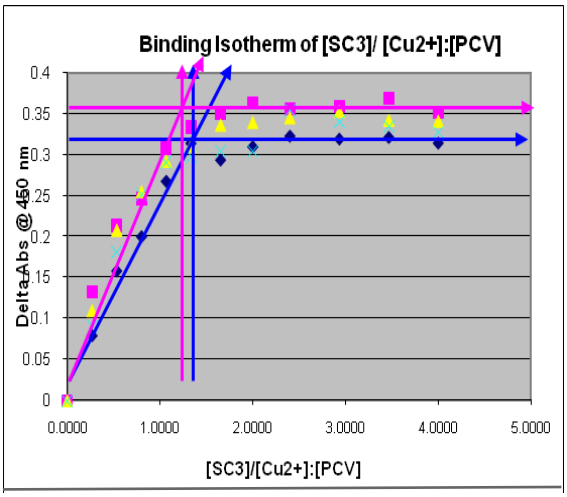


Figure: 3.13(a) Binding Curve SC3

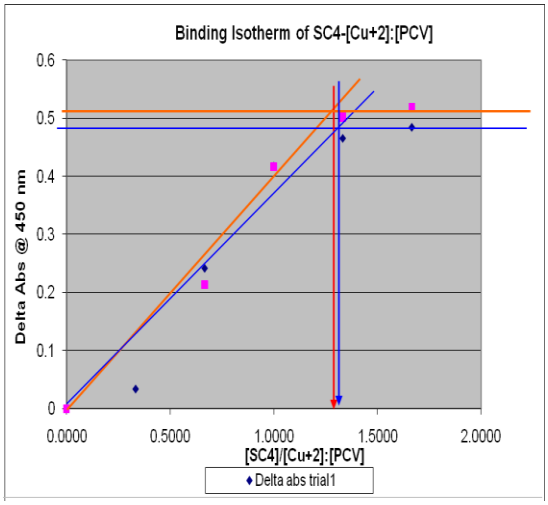


Figure: 3.13(b) Binding Curve SC4

3.5 CABERNET SAUVIGNON WINE SELECTION

UC Davis harvested 17 wines in 2006 and 17 wines in 2007, a total of 34 wines. The seven chosen wines for this study came from harvested wines from 2006 due to their tannin levels (mg/L) see table 4. The tannin levels of the chosen wines ranged from 277 mg/l to 897 mg/l. Two wines selected with the lowest tannin levels, three selected in the middle range, and the two wines selected with the highest tannin level were chosen.

Table 4: Wine list

	Tannin Level mg/L	Wine Code	Harvest
1	897	06H466	H4
2	769	06H168	H1
3	578	06H146	H1
4	407	06H218	H2
5	401	06H116	H1
6	298	06H542	H5
7	277	06H322	H3

3.6 AMINO ACIDS – PEPTIDIC SELECTION

The peptides synthesized were chosen due to previous research by Umali, A. P. et al. (2010) (submitted to journal pending publication referenced with author's permission) where the amino acid histidine was in every sensing ensemble that had a considerable discrimination between the commercially available wines that were tested. Most amino acids selected for the peptide sequence were nonpolar except histidine-which is basic and asparagines which is polar-non-ionized amino acid.

The synthesized peptides SC1 and SC2 are both tripeptides that differ only in the N-terminus amino acid by either methionine or histidine respectively. Encircled differences see illustration 3 (a) & (b). SC3 and SC4 are pentapeptides that differ by one amino acid methionine and histidine encircled see Illustration 5 and Illustration 6. The selection of the other amino acids was due to them being nonpolar molecules and/or the lack of research data or the actual amino acid responsiveness.

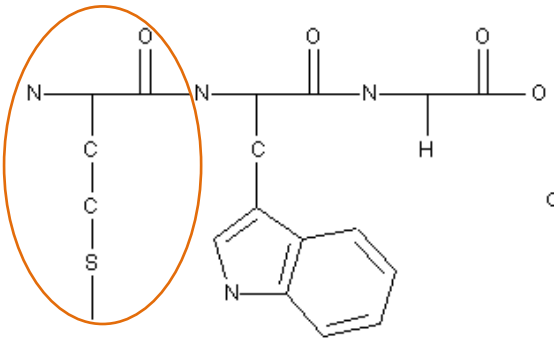


Illustration: 3(a) SC1 – MWG

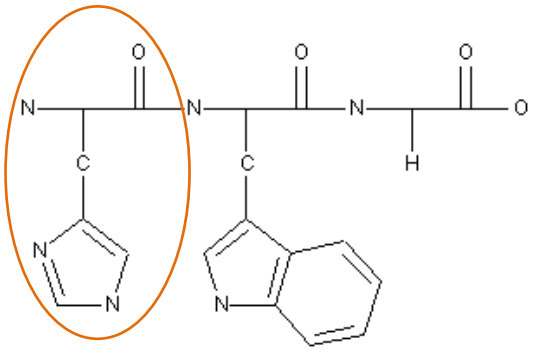


Illustration: 3 (b) SC2 – HWG

Encircled in 3(a) is the methionine versus the histidine in illustration 3(b).

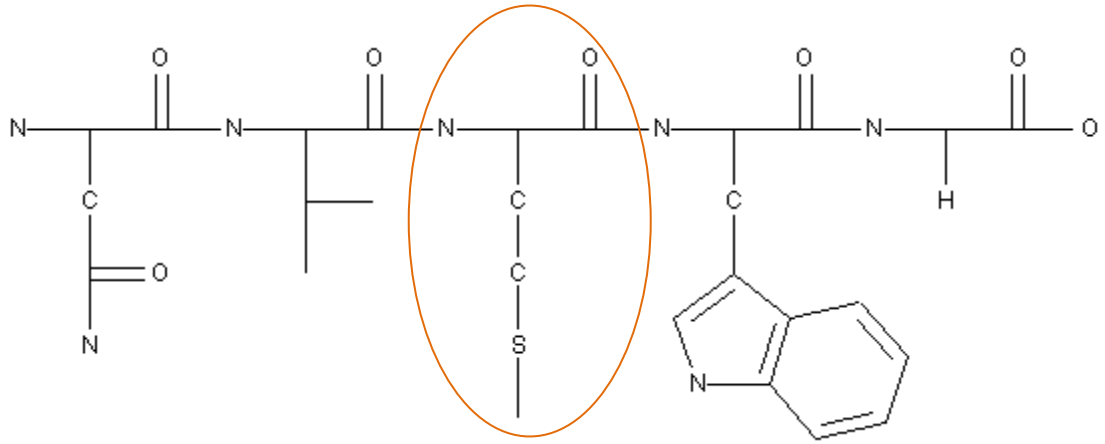


Illustration: 4 SC3 – NVMWG Encircled is methionine

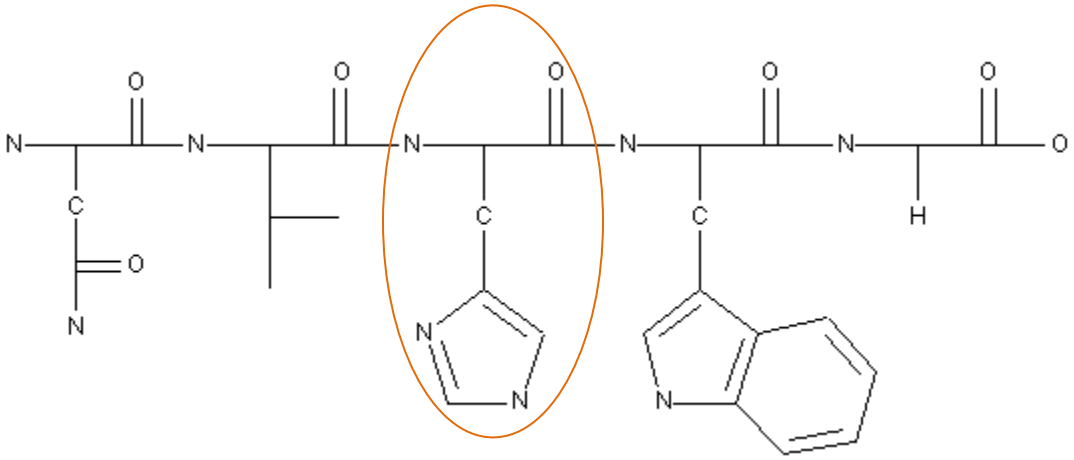


Illustration: 5 SC4 – NVHWG Encircled is histidine.

3.8 WINE ASSAY

The wines samples were prepared to 6% by volume. Then the following solutions were prepared 50mM HEPES in 1:1 EtOH:H_2O at a pH of 7.4, 0.75 mM PCV, 0.75 mM Cu^{2+}, and peptides see Figure 3.14. A multichannel pipette was used. Each selected wine was tested with the polypeptides – LT1, RN8, SC1, SC2, SC3, SC4, and TT2. Some of the peptides were premade. The Molecular Devices SpectraMax® Plus^{384} -

microplate spectrophotometer was used see Figure 3.15(a) and Figure 3.15(b). The data was retrieved and copied on to an excel file where XLSTAT software program would interpret the data collected.

Wine Trials 2

Well plate column Trial	Stock PCV, µL	Stock Cu+2, µL	Stock LT1, µL	Stock RN8, µL	Stock SC1, µL	Stock SC2, µL	Stock SC3, µL	Stock SC4, µL	Stock TT2, µL	Stock Wine Sample 6%	HEPES, µL	[peptide], mM
1 Cu2+:PCV	50	50								50	150	0.010
2 LT1	50	50	50							50	150	0.010
3 RN8	50	50		50						50	150	0.010
4 SC1	50	50			50					50	150	0.010
5 SC2	50	50				50				50	150	0.010
5SC3	50	50					50			50	150	0.010
6SC4	50	50						50		50	150	0.010
7TT2	50	50							50	50	150	0.010
										50	300	0.010
Blank										50	300	0.010

Figure: 3.14 Wine assay

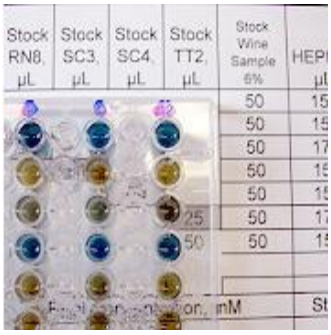


Figure: 3.15(a) Well plate

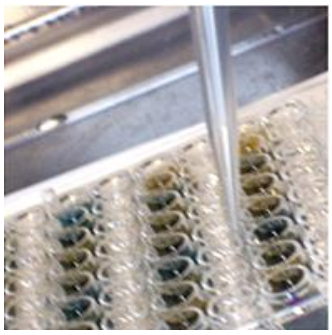


Figure: 3.15(b) Sample well plate

Figure: 3.16 Molecular Device SpectraMax® Plus ^{384} with wellplate

CHAPTER 4 RESULTS AND DISCUSSION

4.1 – RESULTS AND DISCUSSION

The results of the assays with the Cabernet Sauvignon wines harvested at different times revealed that trial one discriminated the wines with utilized six peptide assemble. The six peptide assembles used were SC3, SC4, RN8, TT2, LT1, and [Cu^{2+}]:[PCV]with no peptidic ensemble see Figure 4.1. The correlation circle in Figure 4.1 shows that [Cu]:[PCV] and SC3 they are significantly positively correlated, as are TT2 and SC4. RN8 in comparison with Copper:PCV and SC3 as well as TT2 and SC4 was negatively correlated. PCA loading plot distinguishes between wine 1 and wine 6, the other wines 2,3,4,5 were too close together to be discriminated amongst each other see Figure 4.2.

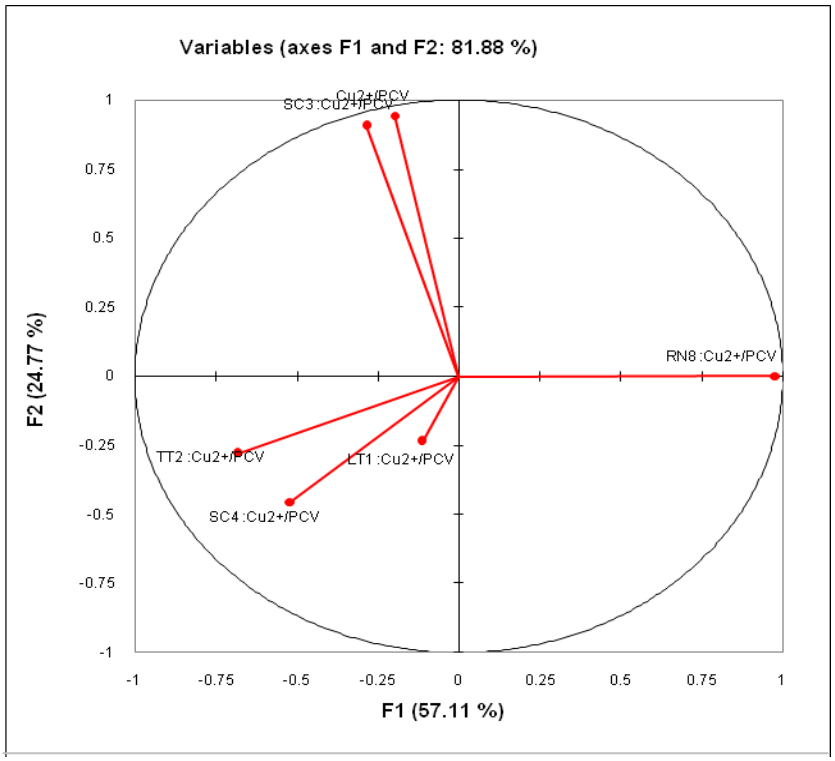


Figure: 4.1 Correlated Circle

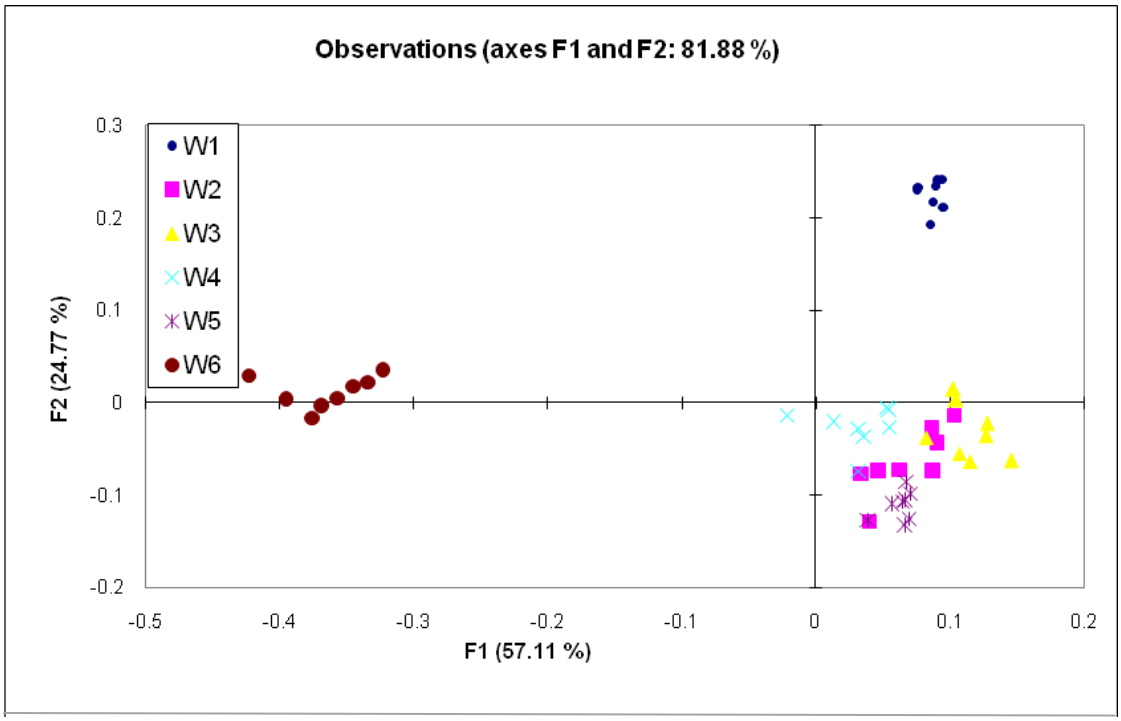


Figure: 4.2 PCA loading plot

In the figure above, the abbreviation were designated as: W1 = 06H466, W2 = 06H168, W3 = 06H218, W4 = 06H116, W5 = 06H542, W6 = 06H322. The greatest discrimination occurred between W1 – 06H466 and W6 – 06H322 were discoved, hence the highest and lowest tannin levels were differentiated between.

In trial 2 and trial 3 consisted of the 7 wines with the sensing ensemble. The biplot for trial 2 shown in Figure 4.3 and Figure 4.4 shows the correlated circle both figures depict the result with the blank consisting of only 50mM of HEPES only. Trial 3 is shown in Figure 4.5 and Figure 4.6 both figures are the results obtained with the blank being HEPES and its respective wine. If Figures 4.3, 4.4, 4.5 and 4.6 they relatively overlap each other, showing that the blank whether composed of wine with HEPES or just HEPES alone does not discriminate samples broadly. Though the overlapping of the

peptides nearly in the same place signifies that they are positively correlated with each other, whether the blank consisted of HEPES alone or HEPES and its respective wine.

Trial 2 consisted of a blank with only HEPES, in Figure 4.4 the results showed that Peptides TT2 and SC4 where located on the bottom right hand quadrant and are significantly positively correlated. Peptides SC1, SC3, RN8, and LT1 were located on the upper right hand quadrant signifying that they are all positively correlated with each other. Tripeptide SC2 was significantly discriminated its position was in the upper left quadrant which signified that it was negatively correlated SC4 and TT2 as they are exactly opposite from each other on the correlation circle. The two biplots shown below depict the wines and the peptides and their relative location. Figure 4.3 had axes calculating 78.78%. Wine W5, W4, and W7 were significantly discriminated from each other, while wines W1, W2, W3, and w6 were located to closely to be significantly discriminated amongst themselves.

Trial three had the blanks consisting of HEPES with their respective wine. Shown in Figure 4.5 had axes calculating 78.07%. The results mimicked the results discussed for trial three.

In terms of answering the question was there a difference in the tripeptides SC1 (MWG) and SC2 (HWG), both peptides ended up on different quadrants on the loading plot, both might be responsible for attracting different wines to them. The pentapeptides – SC3 (NVMWG) and SC4 (NVHWG) did end up on different quadrants, but SC3 did not have wines surround it on the loading plot. SC4 was found to be positively correlated to TT2, hence the wines that near them were due to which peptide, no definitive answer could be given at this time. As for the tannin levels, no definitive trend was seen. There was a definitive difference on 06H401 and 06H218 wines from different harvest and rows, but close range in tannin levels. Wine 06H116 had a tannin level of 401 mg/l and

Wine 06H218 had a tannin level of 407 mg/l. Referring to a Cartesian plane - plotting points wine 06H116 was located on the first quadrant (7, 1) and wine 06H218 was located on the third quadrant (-1.-4) about the same tannin level in both but different harvests.

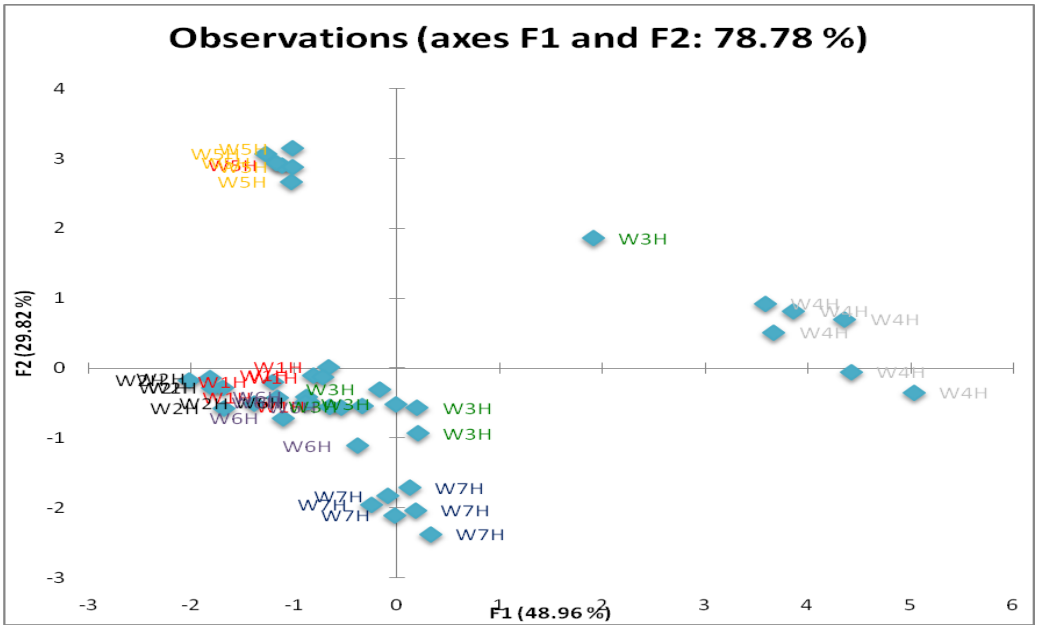


Figure: 4.3 Trial 2 PCA Wines Blank HEPES

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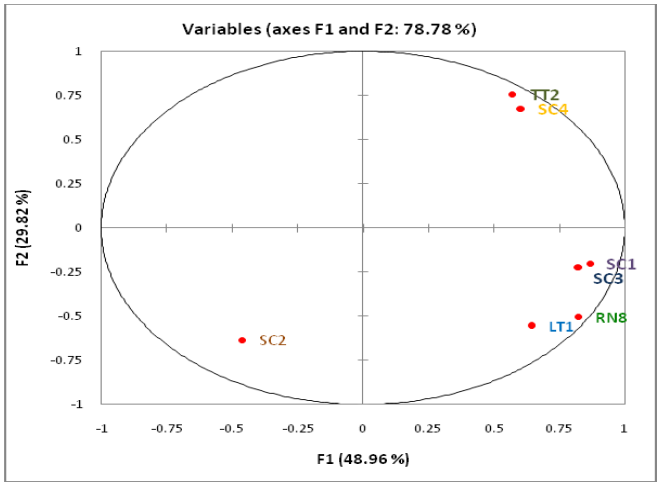

Figure: 4.4 Trial 2 Correlated circle

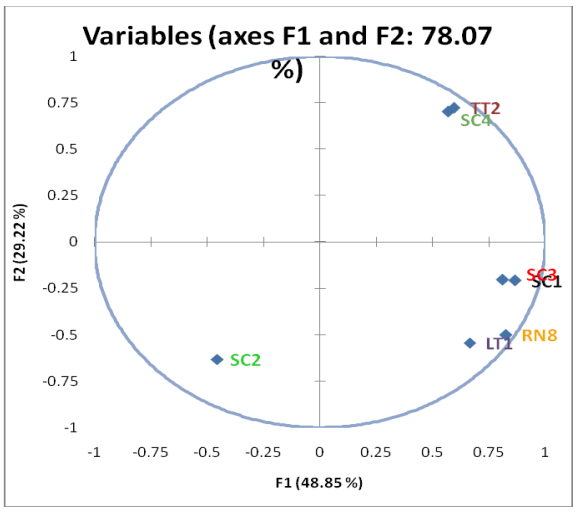

Figure: 4.5 Trial 3 Correlated circle

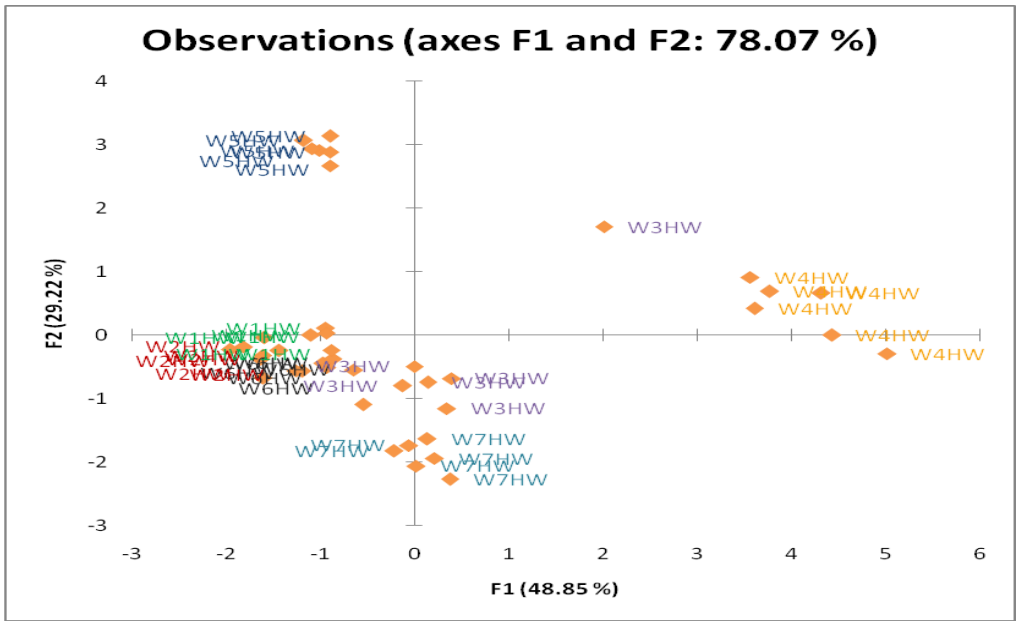

Figure: 4.6 Trial 3 Blank was HEPES with its respective wine

CHAPTER 5 CONCLUSIONS AND FUTURE RESEARCH

5.1 SUMMARY

In summation, the reason behind trying to fingerprint complex molecules is with a bigger vision in mind. Currently identification of complex molecules without evasive procedures has helped to detect heparin, early detection and markers of health issues, dog sniffing out cancer from urine samples.

Tannin levels and flavonoids having some interesting health benefits, if taken in moderation, Tannins comes from plants, grape skin, stems and seeds of grape as well as the oak cask where wine is stored. Monetary interest for wineries and connoisseurs comes into play as well, learning when the tannin level is too high or stopping the grape fermentation before the wine acquires a 'bitter' taste.

One main goal was to discriminate Cabernet Sauvignon wines forming a fingerprint for each different harvest. The time allotted for this research was not sufficient to produce a fingerprint for complex mixtures. However findings have pointed histidine as an indicator that gives greater differentiation and distance from the origin of a correlation circle and the expanding occupancy of the loading plot was achieved.

5.2 RECOMMENDATIONS FOR FUTURE STUDIES

Given the time constraints for this study only two trials were really tested. Could different blanks give us better results? A thorough continual analysis was not successfully achieved. Future studies might include a continuation of these peptides

altering only one amino acid at a time, allocating different reaction time per trials both for introduction of the analyte as well as the host and indicator reaction time, also controlling the temperatures of all solutions, and taking residual Brix values, tannins levels, and maturation of the Cabernet Sauvignon wines in to considerations.

5.3 APPLICATION TO EDUCATION

The research and procedures conducted in this study will be very useful in any science classroom environment.

1. The importance of accurate documentation in their own lab journals.
2. Students will be made aware of the techniques and importance of significant Figures and the role they take on in industry.
3. They could be asked to do an online research project pertaining to how sensing ensembles are used in real life situations and the important roles they will take on.
4. Students will be made aware of the importance of how to conduct a sound scientific experiment along with scientific research.

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Vita

Sally Chong was born in Brownsville, Texas. After completing her high school education at Los Fresnos High School in Los Fresnos, Texas, she enrolled at Texas Southmost College obtaining an Associate of Arts in Liberal Arts in 1995 and earned a Bachelor of Science from the University of Texas at Brownsville in 1999. During the summers she was selected to participate in Fellowships associated with the University of Texas at Brownsville – STEM Summer Fellowship program, University New Mexico – Sandia National Labs, and UTHSC TCOM – Texas College of Osteopathic Medicine in Fort Worth, Texas. During the following years, she was employed as a Laboratory Supervisor at Young Dental Manufacturing before beginning her educational career. Her first teaching assignment was at Raymondville High School in Raymondville, Texas where she taught Integrated Physics and Chemistry during 2000 -2001, Harlingen High School in Harlingen, Texas were she became the chemistry lead teacher, taught chemistry, Pre-AP Chemistry, AP Chemistry, sponsored the Harlingen High School Science Club, and coached the science U.I.L team from 2001 – 2006, and then Homer Hanna High School in Brownsville, Texas were she taught chemistry and Pre-AP chemistry, been a co-sponsor of the Hanna High School Ecology Club, and coached the science U.I.L Team. In June of 2008, she entered the Graduate School at the University of Texas at Austin – UTeach Masters Program to obtain a masters degree of arts in science education with an emphasis in chemistry.

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