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Composición elemental y perfil electroquímico de vinos Chilenos para la determinación del origen geográfico y varietal mediante modelos quimiométricos

Tesis para optar al Grado de Doctor en Ciencias y Tecnología Analítica

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Resumen

Teniendo en cuenta la importancia bioquímica que los elementos tienen en la elaboración del vino, se ha propuesto una caracterización de los principales metales en los vinos de la zona del valle del Itata. Se distinguen dentro de este territorio tres zonas geográficas que son reconocidos empíricamente por los productores. Así como tradiciones vitivinícolas han trascendido a lo largo del tiempo manteniéndose en la actualidad la producción de estos vinos con baja intervención química y tecnológica.

Este estudio se enfocó en el comportamiento de la fracción elemental inmersa en el vino y su potencial para la diferenciación geográfica, recurriendo a técnicas quimiométricas para el análisis de datos multivariados, extrayendo información que relacione las concentraciones de los metales con la calidad y denominación de los vinos. En paralelo se incluyeron estudios electroquímicos no dirigidos para identificar y diferenciar de forma rápida el tipo de cultivar. Específicamente los vinos fueron seleccionados a lo ancho del valle del Itata y la clasificación se centra en las características geomorfológicas de este a oeste. Las muestras corresponden a vinos campesinos, elaborados con metodologías tradicionales y con mínimos tratamientos químicos. La determinación de los componentes inorgánicos del vino, requirió de un espectrómetro de masas con plasma de acoplamiento inductivo o ICP – MS. Técnicas con capacidad de detectar vestigios de metales hasta los niveles de ultra trazas y que, adicionalmente, contienen opciones de lectura de muestra (sistemas automatizados de dilución de muestra y diluciones isotópicas) capaces de contrarrestar las interferencias de la matriz o las causadas por las determinaciones multianalisis. A esto debe añadirse una óptima preparación de muestra que consiga establecer con precisión y exactitud las concentraciones. En el caso del vino, esta matriz es

reconocida por su complejidad para el análisis, al coexistir con una gran cantidad de compuestos distribuidos en una suspensión hidroalcohólica, los que constituyen aproximadamente un 3% del total de la fracción total de compuestos diferentes al agua y al etanol. Estos a su vez se organizan por el nivel de concentración en que se encuentran en el vino. De forma complementaria se incluye el desarrollo de una metodología exploratoria para la determinación de complejos de bajo peso molecular (LMWC), empleando cromatografía líquida con detección UV y detección por espectrometría de masas con plasma de acoplamiento inductivo (SEC/AEC-ICP-MS). Además del estudio *in vitro* de biodisponibilidad para los metales.

Para la elaboración de los modelos de clasificación geográfica y varietal, se desarrollaron modelos de clasificación y predicción con herramientas estadísticas multivariadas y análisis quimiométrico. Los métodos de clasificación y predicción utilizados incluyeron técnicas de exploración como análisis de componentes principales (*PCA*) para realizar análisis exploratorios y técnicas con más rigurosidad matemática y estadística con las cuales se calcularon los porcentajes de clasificación y error sobre un porcentaje de predicción, entre ellos Modelado Suave Independiente de Analogías de Clase *(SIMCA)* y Análisis Discriminate Lineal *(LDA).* Por otro lado, se realizó la investigación descriptiva aplicada a los perfiles electroquímicos de diferentes cultivares de vinos por medio de un arreglo de sensores. Para generar modelos de clasificación con técnicas electroquímicas, fue necesario el uso del Análisis Paralelo de Factores *(PARAFAC).*

1. Introducción

1.1. Origen y variaciones de los elementos inorgánicos en el vino

La fracción inorgánica del vino tiene una naturaleza indiscutiblemente multifactorial donde la relación entre los diferentes niveles de concentración de los elementos dentro de la matriz, las sinergias tanto positivas como negativas y cuya dinámica interna en cada vino ofrecen un patrón característico a cada cosecha, viña y bodega según lo reportado por diversos autores [1–9]. Para poder interpretar el origen y la dinámica de estos elementos se han realizado diferentes categorizaciones de las fuentes que proveen al producto final de la uva.

Un resumen de esto se encuentra esquematizado en la Figura 1- 1. En ella se destacan diferentes clasificaciones para las fuentes de metales en vinos. Es importante destacar la compleja relación entre los elementos del suelo y la planta, así como la influencia de los procedimientos de vinificación, elaboración y almacenamiento en torno a las modificaciones que se presentan hasta el producto final embotellado [3,10–17].

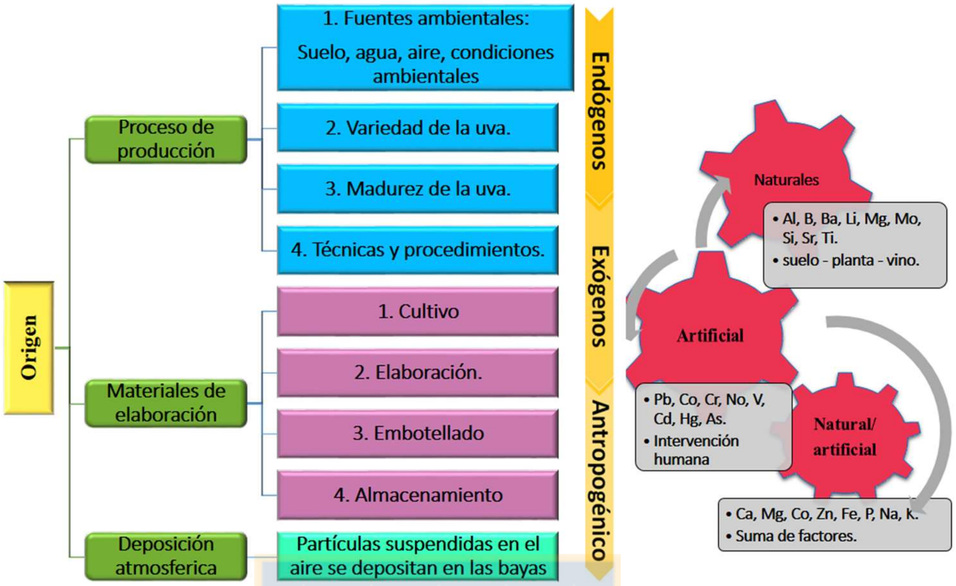

Figura 1- 1. Las fuentes de metales se encuentran desde la cosecha hasta el producto final.

Aspectos como la formación del suelo donde se encuentran plantadas las vides, su historia, factores ambientales, cambios climáticos, geología, así como las levaduras, prácticas vitícolas, transporte, proceso de vinificación y almacenamiento de la uva, el mosto y el vino, se encuentran incluidos en la formación de un perfil elemental [12].

Se considera como fuente directa de elementos en el vino a los factores ambientales, las condiciones naturales del entorno, las propiedades fisicoquímicas del suelo, así como la fisiología de la planta. Principalmente porque finalmente se depositan en la baya, sobre todo en las partes sólidas (pieles 1 – 2%, paredes celulares de la pulpa 0.2 – 0.4%, semillas 1 – 2%) y el raspón [18]. Sin embargo, la intervención humana y/o la contaminación ambiental, las prácticas agrícolas (uso de fertilizantes, pesticidas inorgánicos etc.) y los tratamientos vinícolas, incluyendo productos químicos (en las etapas de prensado, preparación del mosto, pre aclaración, fermentación alcohólica y maloláctica, trasiego, terminación, estabilización, filtración, envejecimiento, embotellado y almacenamiento), y los materiales de los utensilios de vinificación constituyen la fuentes de variación más características de metales en el vino y se considera que estos aportan la mayor parte de los elementos traza [1,19]. En relación a los materiales utilizados en la vinificación, hay que destacar que en la actualidad se han realizado esfuerzos por reducir esta fuente de metales, incorporando materiales como plástico entre otros [19].

En algunos casos la disminución de tratamientos químicos es parte de las estrategias para controlar la carga metálica. Sin embargo, la directa relación entre el vino y el suelo es ambigua [3], por la modificaciones que se realizan en los factores anteriomente mencionados de una bodega o viña a otras ubicadas en una misma zona.

Hopter et al., 2015, ha realizado una resumen, donde se puede ver a los elementos modificados por las prácticas de viticultura y / o vinificación. En la Figura 1- 2 se muestra los elementos en los llamados elementos "naturales" (Al, B, Ba, Li, Mg, Mo, Si, Sr, Ti, elementos de tierras raras (REEs), elementos de transición del segundo y tercer período), elementos "artificiales" que resultan de la intervención humana (prácticas vitícolas, procedimientos de vinificación, contaminación ambiental, por ejemplo, Pb, Co, Cr, Ni, V, Cd, Hg) y elementos que son tanto "naturales" como "artificiales", y que resultan de procesos endógenos o exógenos (*p. ej*. Ca, Mg, Co, Zn, Fe, P, Na, K).

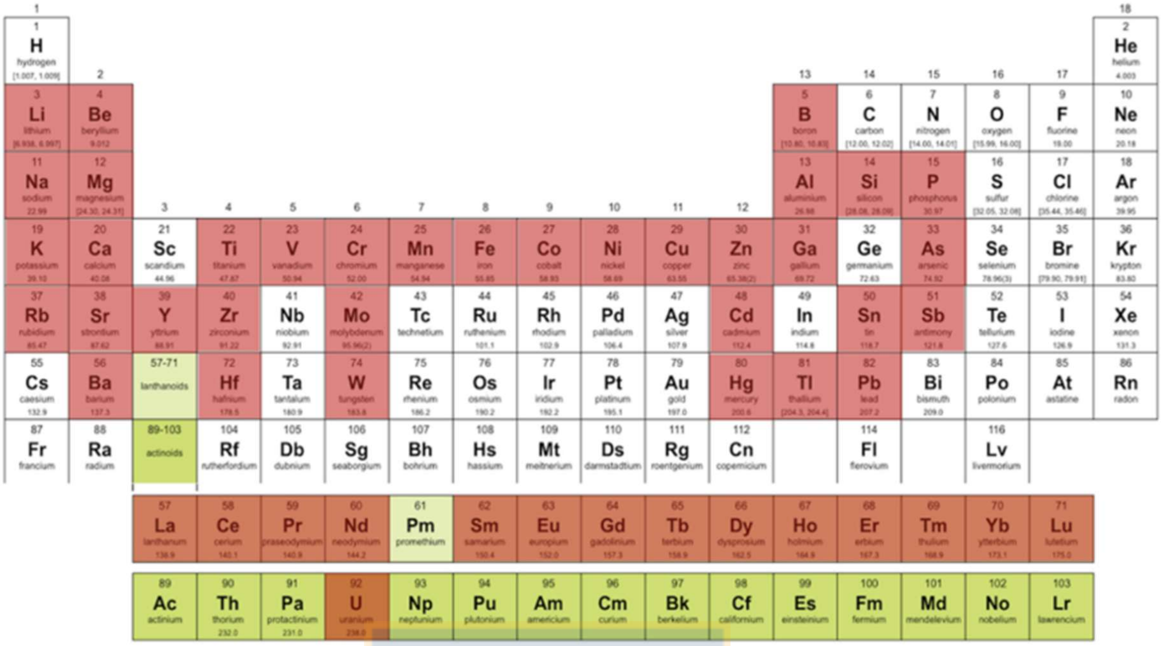

Figura 1- 2. Elementos que pueden variar en función de las prácticas de viticultura y / o vinificación. Rojo (elementos naturales/artificiales). Naranjo (elementos artificiales). Verde (naturales).

También, se estableció un resumen del origen de los metales que afectan la química del vino y las fuentes más importantes que alteran esta carga metálica, ver

Tabla 1- 1 y STabla 1- 1. En esta se analizan específicamente los elementos más significativos en los cambios de las características organolépticas del vino, relacionados con el proceso de vinificación y las condiciones ambientales, que afectan las concentraciones finales de estos elementos.

Tabla 1- 1. Orígenes de los metales que afectan la química del vino y las propiedades sensoriales.

Metal	**Origen**
Al	Suelo (V) [a], bentonita (W) [a], aleaciones metálicas (W), medios filtrantes (W)
Ca	Suelo (V), fertilizantes (V), fungicidas (V), bentonita (W), desacidificación (W), agentes clarificantes (W), filtro medios (W), tanques de concreto (W)
Cu	Suelo (V), fungicidas (V), fertilizantes (V), pesticidas (V), aleaciones metálicas (W), agentes clarificantes (W), estabilizadores (W), medios de filtro (W)
Fe	Suelo (V), aleaciones metálicas (W), bentonita (W), medios filtrantes (W), agentes clarificantes (W), suplementos de levadura (W), estabilizadores (W)
K	Suelo (V), fertilizantes (V), agentes clarificantes (W), estabilizadores (W), Meta bisulfito de potasio (W)
Mg	Suelos de Mg (V), bentonita (W), tanques de concreto (W), agentes clarificantes (W).
Mn	Suelo (V), fungicidas (V), fertilizantes (V), pesticidas (V), medios filtrantes (W)
Na	Irrigación(V), ambiente costero (V) [b], suelo (V), bentonita (W), Meta bisulfito de sodio (W), estabilizadores (W), agentes clarificantes (W), intercambio catiónico (W)
Zn	Suelo (V), fungicidas (V), fertilizantes (V), pesticidas (V), aleaciones metálicas (W), agentes clarificantes (W), estabilizadores (W), medio filtrante (W), suplementos de levadura (W)

[a] Viñedo, (V); enología, (W).

[b] Concentraciones mejoradas, presumiblemente debido a un mayor contenido de Na en el suelo, pero con un efecto potencial del contacto directo de las bayas con el aerosol salino (derivado del aerosol del mar).

Por otro lado desde el punto de vista químico-analítico, el vino es una matriz compuesta principalmente de agua, etanol, ácidos orgánicos e hidratos de carbono [20,21]. En la Tabla 1- 2 se muestra un resumen de sus principales componentes y concentraciones, mientras que en la Figura 1- 3 se representan los porcentajes de distribución.

Tabla 1- 2. Principales componentes encontrados en el vino [21].

Composición del vino			***Concentración***
Compuestos orgánicos volátiles		Etanol	8 -19 % (v/v)
Compuestos no volátiles	Alcoholes no volátiles	Glicerol, Butiletilglicol	1- 10 g l^{-1}
	Azucares	Glucosa, fructuosa, galactosa, manosa	1-20 g L^{-1}
	Ácidos orgánicos/ sales	Tartárico, málico, cítrico, acético	1,8 g L^{-1}
	Otras sustancias	Aminoácidos, polifenoles, flavonoides, Flavonas, etc.	<1g L^{-1}
Sales inorgánicas		Cl-, PO_4^{3-}, SO_4^{2-}, SO_3^{2-}	>10 mg L^{-1}
Elementos	Mayores	Na, Ca, Mg, K.	>10 mg L^{-1}
	Trazas	B, Al, Mn, Fe, Cu, Zn, Sr, Rb	0,1 mg L^{-1}
	Utratrazas	Li, Sc, Ti, V, Cr, Co, Ni, As, Se, Mo, Ag, Cd, Sn, Sb, Ba, tierras raras, Hg, Tl, Pb, etc.	<0,1mg L^{-1}

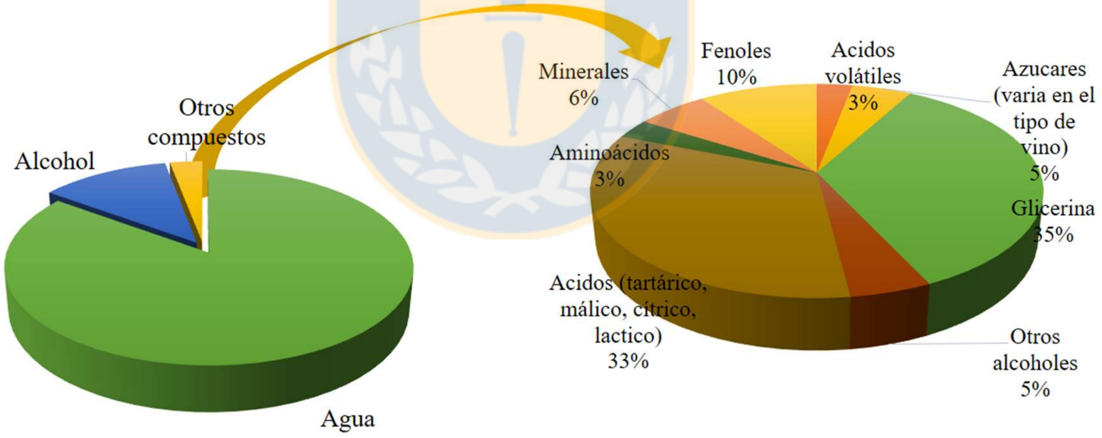

Imagen 1. Componentes mayoritarios del vino

Imagen 2. Otros compuestos del vino (3% del total)

Figura 1- 3. Distribución de los componentes mayoritarios y minoritarios en vino (adaptado de Brendagh 2013).

La clasificación de los elementos se realiza teniendo en cuenta las concentraciones y sus propiedades: elementos mayores, menores (oligoelementos) y elementos traza. Los elementos mayores alcalinos (K y Na) y alcalinotérreos (Ca y Mg) están presentes en concentraciones desde 10 mg L^{-1} para Na, 1 g L^{-1} para el K y del orden de 100 mg L^{-1} para Ca y Mg. Los oligoelementos, o elementos menores son suministrados por la roca madre al suelo en el transcurso de la evolución podológica, en la cual la degradación fisicoquímica fragmenta la roca y libera sus constituyentes bajo una forma más dividida y así biodisponible a las raíces de las plantas. Todos los elementos del sistema periódico que se encuentran en los mostos y en los vinos pueden considerase como oligoelementos. También se han identificado metales pesados como el plomo (Pb), el cadmio (Cd) y el níquel (Ni) en el vino; las concentraciones de estos y otros metales pueden estar reguladas internacionalmente debido a la calidad del vino y las preocupaciones sobre la salud (toxicidad). En los mostos y los vinos, según la OIV están presentes: As (0.2 mg L^{-1}), Cd (0.01 mg L^{-1}), F (1 mg L^{-1}) y Pb (0.2 mg L^{-1}) (Flanzy, 2000). En la STabla 1- 2, se incluyen límites y intervalos de concentraciones permitidas por la Organización Internacional de la Viña y el Vino (OIV) y trabajos donde fueron determinados los niveles de algunos metales [2,8,12,17,22]. Estudios relacionados demuestran que la presencia y concentración de oligoelementos refleja la geoquímica del suelo en el cual las vides son cultivadas, así como la influencia de la intervención humana y del medio ambiente [18]. No obstante, como se ha descrito en párrafos anteriores la dispersión de las concentraciones, producidas por sustancias químicas utilizadas en la viticultura, la contaminación ambiental y la mala práctica de bodega estan estrechamente vinculados al contenido total de los metales en el vino [16].

1.2. Efecto biológico de los metales en el vino

El análisis de ciertos elementos es importante por el efecto que parecen tener en las propiedades organolépticas del vino, pero también por su toxicidad crónica en el caso de una ingesta excesiva y el impacto a la salud [23]. Para los elementos Cu, Zn, Fe Al, As, Cd y Pb se describen estos aspectos en la STABLA 1- 3 [7,19]. Los metales influencian el crecimiento de la vid, la calidad de la cosecha y los parámetros compositivos y sensoriales del vino [2]. Conforme a sus propiedades químicas, permanecerán en forma de metal libre a lo largo de la fermentación, elaboración y envejecimiento del vino o en otros casos formaran compuestos de coordinación, como complejos participando en la vida del vino como catalizadores de importantes reacciones biológicas [24]. Como catalizadores de algunas enzimas, actuando en procesos redox que son requeridos por el metabolismo de la célula, así como estabilizadores de otras moléculas para mantener control sobre su aspecto, color, claridad y sabor [25].

Algunos elementos son necesarios para el crecimiento de las uvas ya que el mosto del vino es un sistema vivo que depende de nutrientes para su elaboración. Las levaduras necesitan algunos nutrientes para su desarrollo y muchos metales cumplen un rol estimulante en el proceso [15]. Un alto contenido de elementos traza parece estar relacionado con los vinos con más cuerpo, altos en acidez, bajo contenido de alcohol y mayor estabilidad durante el envejecimiento [3,15,16].

Entre las reacciones más importantes en el vino vinculadas a metales se encuentra la de óxido- reducción (Redox), [debido a la acción catalítica del Cu, Fe y Mg]. Esta reactividad se relaciona con los potenciales de reducción de los metales y el oxígeno [17,26]. Afectada por los valores de pH, la presencia de compuestos fenólicos y antioxidantes (como SO_2 y ácido ascórbico) ligados como iones carboxilato y agua. En general las reacciones redox son favorecidas por metales, ya sea como pareja redox (*ej.* Fe^{3+}/Fe^{2+}) de forma que los compuestos fenólicos se oxiden y el O_2 se reduce para generar otros oxidantes más potentes (radicales de reactividad variable). Los metales de transición en sus estados reducidos (como Fe^{2+} o Cu^{+}) pueden acelerar la reacción del oxígeno y algunos de los componentes del vino catalizando la formación de especies reactivas del oxígeno (ROS). Los metales que promueven la formación de ROS producen un radical hidroperoxilo (HOO·); y mediante la reacción de Fenton del peróxido de hidrógeno (H_2O_2) produce un hidroxilo altamente reactivo (·OH) [19].

Los estados de oxidación requeridos de los catalizadores metálicos se generan a través del ciclo redox, que promueve efectivamente la oxidación fenólica y la oxidación de ROS.

La presencia de múltiples metales de transición puede tener un efecto sinérgico en el ciclo redox en formación de componentes volátiles y no volátiles. En tanto los compuestos fenólicos pueden actuar como ligandos que se unen a iones de metales de transición influyendo en la aceleración o retardo de los procesos oxidativos. También pueden afectar el color del vino tinto formando complejos con antocianinas (y otros co-pigmentos), (metaloantocianinas) y reaccionar con el azufre formando di y trisulfuros [19].

1.3. Estudios sobre determinación geográfica de vinos por medio de carga metálica

Entre los países que han implementado patrones de perfiles metálicos en estudios dirigidos a la clasificación se encuentran algunos de los principales productores mundiales de Europa, Africa y Sudamérica, los que han desarrollado modelos quimiométricos cuyas técnicas de reconocimiento de patrones más usadas son: Análisis Discriminante Lineal (LDA), Modelo Blando Independiente de Analogía de Clases (SIMCA), K- vecinos más cercanos (KNN) y Red Neuronal Artificial (ANN), con resultados en la clasificación y predicción en un rango de 80–100 % y 74–100% respectivamente [3,8,23,27–29]. Un claro ejemplo de estudios efectuados en Latinoamérica es el que llevo a cabo la Universidad Federal de Santa María en Brasil, donde se estudiaron vinos provenientes desde Argentina, Brasil, Chile y Uruguay, recolectados desde supermercados, obteniendo como factor de selección la concentración de metales como Li, Mg, Rb, Tl y U, cuantificados mediante espectroscopia de emisión óptica con plasma acoplado inductivamente (ICP-OES, *inductively coupled plasma – optical emission spectrometry*) y espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS, *inductively coupled plasma mass spectrometry*) [8]. Otro importante estudio realizado por la Universidad Friedrich Schiller of Jena (Alemania) determinó 19 elementos (As, Be, Co, Cs, Ga, Li, Nb, Ni, Rb, Te, Ti, W, Y, Zr, Mo, Cd, Sb, Tl, U) y tierras raras mediante ICP-MS, logrando un 88% de predicción mediante validación cruzada [29].

En Chile, las investigaciones en este ámbito pueden encontrarse en los trabajos de Villagra et al [26] cuyos estudios se basa en la discriminación varietal de vinos blancos y tintos comerciales del valle del Elqui y el valle del Itata, en las variedades Chardonnay, Sauvignon blanc, Cabernet Sauvignon, Carménère**,** Pinot noir y Syrah determinados por espectrometría de masas con transformada de Fourier (ESI FT MS). Los resultados del porcentaje de varianza acumulada muestran para vino blanco 97% y 85 % vino tinto en el análisis de componentes principales (PCA), es decir, para el vino blanco existe menos variabilidad en las muestras y por tanto la mayor parte de la información suministrada por los datos se recoge en los dos primeros componentes principales al igual que para LDA cuyos resultados fueron 100% y 96% de clasificación (blanco y tinto respectivamente). Concluyen de esta forma que debido a que el vino blanco se constituye de menos compuestos que el vino tinto, es factible obtener menores interferencias a partir de estas muestras y, por ende, mejores resultados en los modelos. En otro estudio Laurie et al [26], determinaron por espectrometría de absorción atómica, la relación entre los elementos mayoritarios (K, Mg, Ca, Na, Fe y Zn) analizando vinos comerciales Chardonnay, Sauvignon blanc, Cabernet Sauvignon, Carménère, Pinot Noir y Syrah de las regiones de Elqui, Limarí, Casablanca, Maipo, Colchagua, Maule e Itata. Además, evaluaron las correlaciones entre los metales a través del origen geográfico, señalando que se logró diferenciar la región del Elquí por el contenido de Na. La validación de estos resultados utilizando estudios cruzados arrojó porcentajes de clasificación correcta de 76% (Blanco) y 62% (tinto), concluyendo que se requiere la construcción de modelos matemáticos más robustos y precisos. Saavedra et al, estudiaron los vinos Pinot noir del Valle de Casablanca, realizando una identificación de las zubzonas a partir de productos no comerciales y sus perfiles metálicos (ICP-OES), permitiendo obtener adecuados *fingerprints* para la clasificación (77%) [30].

Aparte de los antecedentes ya descritos, se puede ver en la literatura que los elementos son determinados generalmente por medio de ICP –MS [2], ICP-OES [4] (y espectroscopia de Absorción Atómica (AAS) [4,29].

1.4. Métodos de determinación de elementos en vino

Los instrumentos de espectroscopia de absorción atómica (AAS) pueden determinar sólo uno o unos pocos elementos de forma simultánea. Aunque la técnica de espectroscopia de absorción atómica electrotérmica (ETAAS) no es adecuada para la determinación con varios elementos, ofrece una alta sensibilidad y selectividad para la determinación de niveles > 1 mg L $^{-1}$ [31]. Entre las técnicas más versátiles para la determinación multielemental en muestras de vino se destacan el ICP-OES e ICP-MS, proporcionando alto poder de detección, alta selectividad y alta sensibilidad [21]. Debido a la alta temperatura del plasma, se observan menos interferencias de la matriz [32].

1.5. Descripción del área de estudio

1.5.1. *Ubicación*

De acuerdo al decreto Nº 464 de la legislación vitivinícola del país, del 14 de diciembre de 1994, Chile cuenta con una zonificación vitícola geográfica, o denominación de origen, basada en valles transversales, generalmente delineados por ríos importantes que fluyen de cordillera a mar. En Chile indicación geográfica y la denominación de origen a menudo calificadas como un signo distintivo que identifica un producto como originario del país o de una región o localidad del territorio nacional, cuando la calidad, reputación u otra característica del mismo sea imputable, fundamentalmente a su origen geográfico. Una Indicación Geográfica hace referencia al lugar o región de producción, extracción, cultivo o elaboración que determina las cualidades específicas del producto originario de dicho lugar o región. Es importante que las cualidades y la reputación, calidad u otra característica del producto sean atribuibles a dicho lugar. Habida cuenta de que dichas cualidades dependen del lugar, cabe hablar de vínculo específico entre los productos y su lugar de producción original. Está zonificación divide al país en cinco grandes regiones vitícolas (Atacama, Coquimbo, Aconcagua, Valle Central y Región Sur) y 15 subregiones.

El valle de Itata es una subregión dentro de los tres valles perteneciente a la región vinícola de la región sur, junto a el valle de Bío-Bío y Malleco [33]. El territorio comprendido por el valle del Itata consta de una superficie continental de 3.660 km^2 o

311.418 ha, corresponde a un 9.9% de la superficie regional y al 28% de la superficie de la Provincia de Ñuble. En la Figura 1- 4, se muestra el mapa del territorio comprendido por este valle.

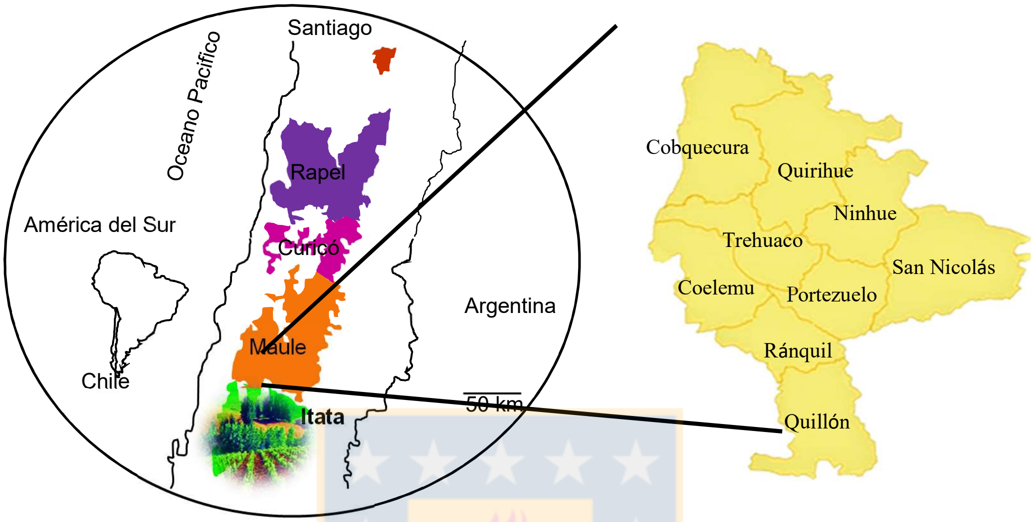

Figura 1- 4. Ubicación geográfica del valle del Itata. Adaptado de [34]

Además tiene un total de 13,030 hectareas representando un 9.2% del total de hectáreas para la producción de vino. Entre las singularidades del valle de Itata se destaca la presencia de cultivares patrimoniales (País, Moscatel de Alejandría y Cinsault) siendo estos los más relevantes en términos de hectáreas en comparación con el resto de valles, con alguna excepción en el valle del Maule, donde predominan los cultivares tradicionales franceses (Cabernet Sauvignon, Sauvignon blanc, Merlot y Chardonnay). Sin embargo, a diferencia de otros valles con mayor tradición comercial, la producción del Valle del Itata se ha destinado principalmente para obtención de vinos a granel [35] para hacer vino de mesas o vender uvas sin denominación de origen y mezcladas con uvas de otras regiones vinícolas. En la actualidad, se encuentra como un mercado emergente donde el valor agregado de sus productos destaca por pertenecer a la categoría de vinos orgánicos [35].

El territorio del valle de Itata se relaciona con la cuenca del Río Itata que junto a sus afluentes lo cruza de este a oeste. Se distribuye sobre la denominada depresión intermedia, la cordillera de la costa, con altitudes relativamente bajas (400 msnm) y disectada en dos cordones que rodean la cuenca de Quirihue y sobre las plataformas litorales de poca amplitud (menos de 5 km de ancho). La presencia de la Cordillera de la Costa provoca cierta diferenciación topoclimática entre su vertiente occidental con mayor cantidad de precipitaciones y su vertiente oriental con mayor aridez [35].

1.5.2. Entorno geomorfológico y geológico

Chile tiene cuatro macroformas geográficas que varían según la latitud y son clave para comprender las variaciones geo-climáticas del país. Comenzando desde el este y extendiéndose hacia la parte occidental del país, las macroformas incluyen: la cordillera de los Andes; la depresión intermedia; las montañas de la costa; y, finalmente, las llanuras costeras [36].

Por otro lado, el suelo encontrado en este valle se deriva principalmente de la cordillera costera generada a partir de tres diferentes materiales parentales, tales como

1. Roca metamórfica erosionada: muestra presencia de pizarra, arenisca, filita y pizarras; textura del suelo arcilloso e infiltración lenta de agua. Este tipo de suelo normalmente se encuentra en posiciones altas en áreas con una topografía que presenta colinas empinadas o pendientes variables y complejas, que exhibe la formación de Catena debido a la topografía y las características de drenaje.

2. Origen granítico: ha sido derivado de roca granítica y diorita, que presenta texturas arcillosa e infiltración de agua baja en una topografía de colinas con pendientes variables y complejas que hacen que el suelo sea susceptible a la erosión hídrica
3. Sedimento aluvial fino: derivado de sedimentos aluviales finos es el resultado de la deposición de grandes cantidades de sedimentos fluvioglaciales de la glaciación de la montaña de los Andes, fenómeno ocurrido durante la era cuaternaria. El espesor del depósito varía considerablemente debido a las influencias de los ríos presentes en la zona que transportan grandes cantidades de sedimentos especialmente del material fino que han formado los suelos de este grupo con una arcilla franco arcillosa y clases de textura de arcilla y drenaje pobre [36].

1.6. Análisis Multivariado

Los métodos de análisis multivariable (AM) se utilizan para estudiar las relaciones que hay entre más de dos variables. El objetivo del análisis de datos multivariados es estudiar, analizar, representar e interpretar a la matriz de datos de estudio con respecto a las variables [37]. La información estadística en AM es de carácter multidimensional, por lo tanto la geometría, el cálculo matricial y las distribuciones multivariantes juegan un papel fundamental [38].

1.6.1. Análisis de principales (PCA)

Los métodos estadísticos multivalentes son una herramienta útil en el estudio y la clasificación de origen de los vinos, aun cuando estos se basan en complejos conjuntos de datos químicos. El PCA es un método de proyección donde se disponen los datos multivariados en un espacio de dimensión más reducido, además de detectar muestras atípicas. Es un método basado en explicar la correlación existente entre los datos, utilizando para ello un menor número de variables involucradas, que las existentes para los datos originales [39].

Estas nuevas variables denominadas factores o componentes principales son combinaciones lineales de las variables originales. Los componentes principales o *"scores"* son básicamente las coordenadas de los objetos en las nuevas variables, complementados con los análisis de "*loadings"* que corresponden a los pesos de las variables originales sobre los componentes principales.

En un sistema XnXm

n: objetos o experiencias, *m*: características o variables. Un primer paso para estudiar el sistema es tomar 2 variables (*k* y *l*) y ver si estas varian conjuntamente en alguna dirección: covarianza.

$$\operatorname{cov}\left(X_k, X_l\right) = \frac{1}{n-1}\sum_{h=1}^{n}\left(X_{h,k} - \bar{X}_k\right)\left(X_{h,l} - \bar{X}_l\right) \text{ Ecuación 1.}$$

Siguiendo este procedimiento para todas las combinaciones de columnas se obtiene la matriz de Varianza-Covarianza R_v

$$R_v = \frac{1}{(n-1)} \cdot X_C^T \cdot X_C \text{ Ecuación 2.}$$

Donde X_c es la matriz X centrada en columnas (a cada valor se le resta la media de la columna)

1.6.2. Modelado blando independiente de analogías de clases (SIMCA, Soft Independent Modeling of Class Analogy)

SIMCA es un método de modelado basado en la distancia no probabilístico introducido por Svante Wold. Los modelos SIMCA se basan en componentes principales (PC), que son, por definición, las direcciones de la varianza máxima (y, por lo tanto, de la información máxima) en un espacio de datos multivariante. Es un método de clasificación supervisado donde cada clase se modela usando un PCA de forma independiente, de forma que cada clase tiene un modelo específico que la describe con el número óptimo de componentes principales [40]. De esta manera se puede construir un "espacio de clases" cuyo volumen marca el límite entre las muestras que son descritas por el modelo como pertenecientes a las clases estudiadas y las muestras que no pueden ser consideradas o que no pertenecen a ninguna de las clases. Para conocer si una nueva muestra se ajusta a una clase, la información que se calcula es dividida en dos, una parte explicada por el modelo de la clase y otra que permanece en los residuales. Si los residuales de la muestra son significativamente mayores que los de la clase, la muestra es rechazada [39,41].

1.6.3. Análisis discriminante lineal (LDA, Linear Discriminant Analysis)

El análisis discriminante es una técnica supervisada de clasificación, es decir, donde se han definido previamente el número de clases y de muestras que pertenecen a cada una de ellas. Cuanto mayor son las diferencias entre las clases dadas, mayor es la distancia de Mhalanobis entre ellas. La discriminación de las muestras se consigue mediante el cálculo de la distancia de Mahalanobis de cada una de las muestras a los centros de los grupos considerados. Una muestra desconocida se clasifica como perteneciente al grupo con el cual tenga una distancia más cercana al centro [27]. Comparando el PCA con el LDA se puede mencionar que el PCA selecciona la dirección que retiene la máxima estructura entre los datos con el que se construyó el hiperplano de trabajo. Mientras que el LDA selecciona la dirección en la que se consigue una separación máxima entre los grupos definidos. En la construcción de este modelo se debe tener en consideración que requiere un mayor número de muestras que de variable [27].

1.7. Hipótesis

1.7.1. Hipótesis General

- El uso integrado de métodos ICP-MS y el estudio de sensores electroquímicos, para la formulación de modelos de clasificación según la composición elemental y la información electroquímica de vinos cosechados y elaborados en zonas con diferencias latitudinales, es decir con cercanías a la cordillera de los Andes o a la cordillera de la Costa, conocida por los productores como denominación este-oeste, permitirá establecer porcentajes de clasificación y predicción cercanos al 100% en vinos, de acuerdo a la origen geográfico y varietal para establecer una firma química y una correlación entre la fuente y su composición.

1.8. Objetivos

1.8.1. Objetivo general

Generar modelos de clasificación con técnicas quimiométricas de análisis multivariado para identificación del origen geográfico y varietal de vinos jóvenes del valle de Itata a partir de los datos generados por medio de ICP – MS y sensores electroquímicos. Además, de realizar estudio sobre compuestos ligados a la fracción metálica en las muestras de vino mediante cromatografía e ICP-MS así como determinar el porcentaje de biodisponibilidad de en vinos chilenos del Valle de Itata.

1.8.2. Objetivos específicos

- Evaluar la fracción inorgánica en las muestras de vinos por medio del análisis de ICP – MS.
- Realizar estudios sobre complejos de compuestos orgánicos de metal en vinos chilenos del valle del Itata, mediante cromatografía líquida e ICP-MS.
- Desarrollar ensayos de biodisponibilidad de los elementos en vinos chilenos del valle de Itata.
- Diseñar un dispositivo electrónico a partir de un arreglo de electrodos no selectivos y realizar la clasificación varietal.
- Ajustar y validar el modelo quimiométrico para la diferenciación varietal y de origen geográfico identificando las correlaciones entre las variables y las clases por medio de análisis de componentes principales PCA, SIMCA y LDA.

1.9. Estrategia analítica

Para estudiar la carga elemental presente en los vinos y desarrollar los modelos de clasificación geográfica y varietal, la estrategia analítica seleccionada fue esquematizada tal como se ve en la Figura 1- 5.

1.9.1. Muestreo

Se recogieron muestras de vino tinto y blanco del valle del Itata, el cual fue seleccionado posteriormente a una investigación realizada a mayor escala en los valles vitivinícolas más representativos de la zona centro y sur. Particularmente el valle de Itata es elegido para este estudio debido a los modelos preliminares investigados reflejaron una tendencia de clasificación mayor. Además cuenta con una interesante geomorfología y una emergente productividad de elaboración de vinos. El enfoque de esta investigación se centrará en cepas características de la zona (viñas y valle) que a su vez serán seleccionadas en función a la denominación geográfica costa, depresión intermedia y Andes.

1.9.2. Determinación de la carga multielemental por medio de ICP-MS

Con respecto al establecimiento del perfil elemental, se estudió y estableció el método multielemental ICP-MS. Validandose la metodología para la preparación de la muestra y el método de análisis.

1.9.3. Ensayos quimiometricos

La exploración de los datos se realizó en primera instancia por métodos clásicos de ANOVA. Sin embargo, fueron requeridos técnicas con mayor capacidad de análisis multivariado y donde PCA, SIMCA y LDA fueron seleccionadas. El primer método se utiliza para reconocer el comportamiento natural de los datos, no categórico. El SIMCA y LDA hicieron parte de la estrategia de clasificación asociado a un nivel de confianza estadístico.

1.9.4. Implementación de ensayos para fraccionamiento de complejos organometálicos de bajo peso molecular

Se agregaron ensayos para encontrar relaciones entre los elementos y los compuestos orgánicos bajo peso molecular en el vino. Inicialmente se optimizaron las condiciones de separación por cromatografía de exclusión por tamaño (SEC), seguidamente fueron recogidas las fracciones correspondientes de esta separación las cuales se inyectaron en una columna AEC para finalmente ser analizadas por ICP-MS.

1.9.5. Ensayos de biodisponibilidad de metales *in vitro*

Adicionalmente se incorporaron metodologías in vitro para determinar la tasa de biodisponibilidad de los metales. Los ensayos realizados para la biodisponibilidad fueron obtenidos por medio de dos etapas de laboratorio (etapa gástrica y etapa intestinal). Se calcularon los índices de biodisponibilidad de estos compuestos a partir de los balances de masa de las fracciones dializas o no dializadas.

1.9.6. Modelo quimiométrico para clasificación varietal a partir de sensores electroquímicos

La evaluación de diversos materiales conductores para realizar los electrodos de trabajo idóneos para generar señales electroquímicas que por medio de técnicas quimiométricas logren dilucidar los patrones de diferenciación por cultivar. Se evaluará directamente sobre el vino las señales voltamétricas y amperométricas que serán requeridas para desarrollar los modelos de diferenciación.

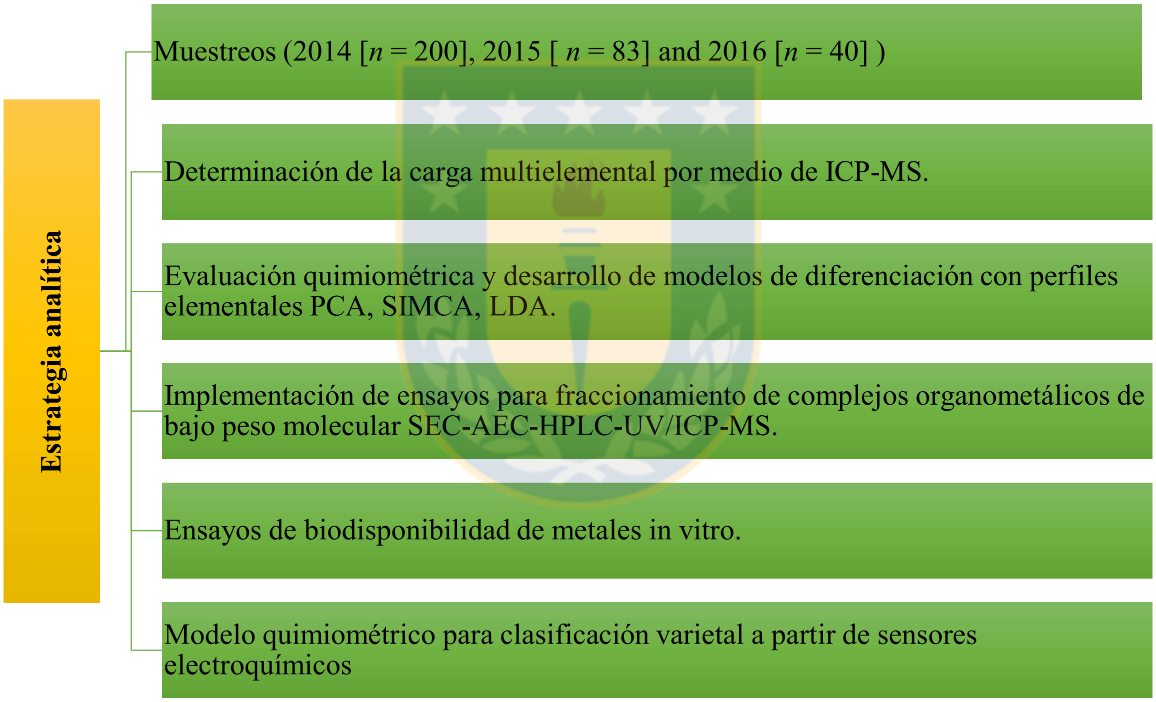

Figura 1- 5. Esquema diseñado como estrategia analítica para el desarrollo experimental.

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Material suplementario

STabla 1- 1. Estados de oxidación, impactos potenciales sobre las propiedades del vino de los metales en el vino y principales fuentes.

Elementos	**Función y/o fuente**
K, P, N	Fuente: Suelo, nutrientes esenciales para la planta, inciden sobre la cantidad de fruta cosechada y calidad del vino obtenido.
Na, K, Ca, Fe, Cu, Zn	Sufren grandes cambios durante todo el proceso tecnológico de elaboración de vino.
Ca, Na, K, Mg	Eficiente fermentación alcohólica, reguladores del metabolismo de la levadura. Mantiene el pH adecuado y balance iónico.
Cu, Fe, Mn, Zn	Son favorables para la activación de metalo-enzimas de las levaduras. Cambios en la estabilidad del vino envejecido. Modificación de las propiedades sensoriales del vino después del embotellamiento (pardeamiento: cascada de reacciones de oxidación de compuestos orgánicos del vino, dirigiéndose a la perdida de frescura y aroma más la aparición de taninos precipitados)
K, Ca, Cu, Cd, Mn, Zn y Pb	Fuente: Fertilizantes (aplicado al suelo o rociado en la vid). Pesticidas, Fungicidas
B, Mo.	Mayor rendimiento.
B, Cr, Mn, Mo, W y Zn	mayor rendimiento, acumulación de azúcares, polifenoles y aminoácidos sobre el suelo de vid o viñedo, por lo tanto, probablemente también se encuentran en el vino resultante
Cr, Mo, W	Introducidos en el suelo en forma de spray, aumenta el rendimiento de la uva y otras sustancias en el vino (azucares, taninos, sustancias colorantes, esteres y aldehídos). Puede aumentar el crecimiento celular de la levadura. Tratamientos con fertilizantes de Cr, promueven el sabor suave.
W, Mn, Co	Incremento del contenido de aminoácidos esenciales.
B, Zn, Mg	Fuente: Aplicados al suelos en N-P-K. Aumento de azúcar en la uva. Disminución de la acidez valorable.
Mg^{2+}	Los mostos con alto contenido, contienen altos niveles de N-aminas y sabor superior.
B, Mn	Acumulación de aldehídos. Crecimiento y desarrollo de la levadura. Mejora el aroma en vino.
Mn, Co, Be, K	Reducción del contenido de azúcar. Reducción de acidez. Reducción de la relación alcohol/acidez del vino.
As	Herbicidas e insecticidas aplicados al suelo. $NaAsO_2$ Aplicado como funguicida para prevenir las enfermedades de las plantas (principal fuente). Componente natural de algunos tipos de suelos. Condiciones de almacenamiento.
Cd, Hg, Pb($^{2+}$, $^{4+}$)	Fuente: Polución antropogénica. Ninguna función. Se asocia a viñedos ubicados cerca de carreteras o zonas industriales. El Pb puede reflejar malas prácticas de bodega, uso de utensilios de bronce para vinificación (bombas, válvulas, grifos y tuberías). El Cd se introduce al vino principalmente por la aplicación de fertilizantes fosfatado.

Tabla continuación

Elementos	Función y/o fuente
Na^{+}	Fuente: Aerosoles marinos
B, Cd, Cs, Ni, Rb, Sn y Zn	Fuente: Bioacumulación. Mayor movilidad por lo tanto, se acumulan en la planta más fácilmente
B, Cd, Mn, Mo, Se y Zn	La acumulación de elementos del suelo también depende en qué tan fuertes son quelados. Estos elementos son débilmente quelatados, por lo tanto, son más fáciles de absorber por la planta.
Ag, Al, Cu, Cr, Fe, Hg, Pb, Si, Sn, Zr.	Otros elementos están fuertemente ligados al suelo o son retenidos en las raíces y no se trasladó a las otras partes de la planta, incluso si está presente en altas concentraciones en el suelo. La absorción de elementos en una planta también depende de las propiedades químicas.
Cu y Ni, S y Se, Cd y Zn. Li y K, Rb y K.	La absorción de elementos en una planta depende de las propiedades químicas, por ejemplo, los elementos no nutrientes comparten propiedades químicas similares con elementos nutricionales por lo tanto estos elementos también pueden ser asimilados por la planta.
Ca, Cu, Fe, K, Mg, Zn	En la etapa de fermentación el contenido elemental en el vino disminuye después de la completa fermentación, debido a la precipitación de complejos de elementos con tartratos, polifenoles, proteínas y azúcares, menor solubilidad elemental en la solución etanólica en comparación con el jugo de uva, las levaduras que consumen elementos durante fermentación (y / o trasiego de sedimentos y precipita al final de la fermentación).
Mn, Zn, Mo	En la etapa de fermentación contribuyen a la aceleración del crecimiento de la levadura. Reducción de la acidez total. Incremento del contenido de alcohol. Aumento de la biomasa.
K $^{1+}$	En la fermentación aumenta la multiplicación y crecimiento de la levadura. En concentraciones correctas favorece a sabor ácido agradable. A concentraciones muy elevadas al sabor amargo y en concentraciones muy bajas el sabor insípido. A media que aumenta la con de K se reduce la del ácido tartárico libre aumentando el pH, mejorando el ambiente de proliferación de bacterias maloláctica.
Ca $^{2+}$	Inhibe el crecimiento de la levadura. En exceso genera sabores indeseables.
Ca, K	Precipitación de las sales de Ca^{2+} y K del ácido tartárico, influyendo en las propiedades gustativas del vino alterando el pH. En el caso de bitartrato de potasio disminuye la acidez valorable.
Na $^{1+}$	No mostró ningún efecto significativo en la fermentación. Por otro lado puede aumentar la salinidad al tiempo que disminuye el sabor agrio y amargo.
Mg $^{2+}$	Ayuda a la separación de sustancias coloidales y otros elementos (Sn y Al (responsables de problemas de turbiedad)). Promueve el sabor ácido. Favorece el buen sabor del vino tinto.
Fe $^{2+}$, $^{3+}$	0 – 50 mg Kg^{-1} no produce ningún efecto en la fermentación. Acumulación de aldehídos después de una vigorosa fermentación. A bajos niveles, soportan el envejecimiento sin volver rancio el vino. Fuentes: Sales ferrosas de ácidos orgánicos (tartárico, fumárico, nítrico), Cataliza reacciones redox en vinos jóvenes. Mejora la calidad organoléptica debido a la oxidación del ácido tartárico y ácido dioximaleico. Cataliza la combinación química de acetaldehído con compuestos fenólicos, en altas concentraciones reduce la cantidad de acetaldehído.

Tabla continuación

Elementos	Función y/o fuente
Cu $^{1+}$, $^{2+}$	Máx. 10 mg Kg^{-1}, produce ligera reducción del porcentaje de alcohol. Aumento de los ácidos volátiles, aumento del sulfuro anhidro en presencia de (Zn y Mn). Actividad con polifenoloxidasa. Procesos microbianos oxidativos. Conduce la formación de H_2S y etanotiol en vinos almacenados bajo condiciones anaerobias (reductoras). Puede formar precipitados insolubles con H_2S y tioles (metatiol y etanotiol). La adición deliberada de Cu^{2+} + $CuSO_4$ (± 0.1 - 0.5 mg L^{-1}) se utiliza para eliminar los compuestos de azufre indeseables (vino blanco) que tienen un impacto negativo sobre el aroma. El tratamiento no es específico y puede afectar las concentraciones deseables de tioles ya sea a través de la unión o como resultado de reacciones redox debido al Cu residual. Bajo condiciones reductoras las concentraciones de Cu (1 mg L^{-1}) pueden conducir a compuestos insolubles con proteínas e inestabilidad de proteínas. El uso intensivo produce fitotoxicidad en la planta y daño en la calidad del vino. Además se relaciona con una mayor tasa de deterioro oxidativos que promueve el oscurecimiento del vino (perdida de frescura, aroma, aparición de precipitados condensados y taninos)
Mn $^{2+}$, $^{3+}$, $^{4+}$, $^{6+}$, $^{7+}$	Fuente: sales de Mn y semillas ricas en Mn. Acelera el proceso de fermentación a una temperatura 40 - 45 °C. Favorece la formación de acetaldehído durante la oxidación lo que explica la influencia del Mn en la desaparición de compuestos fenólicos y taninos. El aumento de concentración y la formación de acetaldehído producen una mayor polimerización y posterior precipitación de compuestos fenólicos.
Zn $^{2+}$	Persistencia del sabor amargo del vino incluso a bajas concentraciones.
Li	Conservación de la fermentación alcohólica. Uso ilegal.
Cr, Mo, W	Efectos positivos sobre la fermentación, aumento de biomasa, impone propiedades organolépticas.
Ag, Zr, Ni, Co	Fuente: Óxidos de cada metal. Utilizados como catalizadores en el proceso de envejecimiento.
Cu, Zr, Ni, Co, Th	Efecto positive sobre la fermentación sobre las propiedades químicas y organolépticas del vino. Incrementa la concentración de 2, 3 – butileneglicol y ácidos volátiles.
Al $^{3+}$	Fuentes: sales de aluminio, conteiner con Al. Defectos organolépticos. El uso de bentonita aumenta la concentración de Al. Provoca opacidad >10 mg L^{-1}
Al, Fe	Cambios desfavorables en el sabor debido a la corrosión.
Al, Zn, Ni, Fe, Cu	Comportamiento similar al Fe^{3+}, Produce a concentraciones mayores 10 mg L^{-1}, turbidez, astringencia sabores indeseables (metálicos, amargos, agrios). Formación de neblina, cambio de color (ya que forman complejos con antocianinas y taninos).
Ni	Promueve el efectivo incremento en el nivel de acetatos.
Ag	Elimina el óxido de azufre del mosto. Utilizado para el envejecimiento artificial. Actúa como inhibidor para la fermentación de ácido acético (sin interferir en la fermentación alcohólica), formando una película que cubre el mosto y evita su exposición al aire.
Fe, Cu	Se adiciona en forma de sales. Produce un aumento en la acumulación de aldehídos, acetatos, esteres, disminución del pH y del contenido de alcohol. En forma libre mejora la estabilidad del color en vinos blancos. Cu^{2+}, Fe^{3+} a 1 -7 mg L^{-1}, se asocia a sabores amargos cúpricos o férrico, astringente desagradable y responsables de la turbidez cúprica o férrica. Especialmente cuando es alto el contenido de sustancias tánicas y/o pH.

Tabla continuación

Elementos	Función y/o fuente
Zn, Mn, K	Favorecen el buen sabor del vino blanco.
Pb	Ningún efecto
Sn	Contenedores de estaño y dióxido de azufre. Responsable de un aroma débil en el vino.
Mn, Be, Co	Estimula la autoxidación del vino con el fin de producir aldehídos y diacetil durante el almacenamiento.
Mo, Zn, B	Produce un efecto inhibidor de aldehídos y cetonas durante el almacenamiento. Si provienen del suelo garantiza la estabilidad del vino durante la maduración y envejecimiento.
Al, Cr, Cd, Co, Fe, Mo, Mn, Ni, Pb, Sr, Ti, V, Zn.	Fuente: diferentes materiales en bodegas, además de los equipos de la bodega incluyendo acero inoxidable, latón, madera y plásticos, conteiner de cobre, barriles de roble, tubos, accesorios y trampas.
Al, Fe y V.	Fuente: Contaminación por el recipiente de guarda en barricas de roble (envejecimiento). Aumentando su contenido.
Pb	En las prácticas de vinificación durante el prensado y la fermentación. Es liberado de pieles y semillas de la uva reflejando niveles más altos de Pb en el vino resultante. Almeida y Vasconcelos (2003b) fueron capaces de establecer el contenido final en el vino en: 1/3 proveniente de suelo inactivo y deposición atmosférica, y 2/3 procedentes la fabricación de vino.
Al, Cu, Fe, Zn, Mn y Sr,	La separación de bayas de uva de los raquis durante la desintegración aumenta los niveles de estos elementos. Pueden tener un efecto negativo, formación de neblina, cambio de color.
Cu, Zn	Con la trituración, se aumentan los niveles.
Al, Cd, Hf, REEs, Pb, U y Zr	Fuente: Aclaración con bentonitas
REE, Co, Cr, Fe y Ni	Fuentes: prácticas de filtración (filtros de sílice, filtros de celulosa, filtros de lechos) Aumenta los niveles
Sn	Factor: envejecimiento y el almacenamiento, la composición elemental del vino puede ser cambiada por materiales utilizados.
Cr	Aumento de los niveles de Sn en botellas con tapa de rosca
REEs	Uso de óxidos de Cr a botellas de vidrio de color REEs más altos cuando se almacenan en botellas de vidrio

***Fuente*:** (Waterhouse et al., 2016), (Hopfer et al., 2015), (Tariba, 2011), (Ibanez et al., 2008), (Rossano et al., 2007), (Flanzy, 2000), (Pohl, 2007), (Pereira, 1988).

STabla 1- 2. Niveles promedio de metales informados en la literatura para muestras de vino.

Concentración promedio / µg L^{-1}							
	OIV. O, 2016	Thiel, 2004	Fabani, 2010	Geana, 2013	Bentlin, 2011	Mercosur regulation	Azcarate, 2015
Li	10 - 50	17.6			3-14		941
Be		1.27		7.29			17
V	3 - 200			47.61	21-281		358
Mn	500 - 1500		0.8	806	430-3270		1778
Co		4.65		4.35	2-12		24
Ni		24.9					276
Cu							186
Ge							6.3
As		7.1			4-79		20
Rb	500 - 1200	349	890.11		1110-5935		531
Sr	300 - 1000		540.55				556
Mo		11.3			2.2-38		10
Cd	1 - 40	0.41			< 0.01-6		7.0 x 10^{-1}
Ba							29
Tl							6.5 x 10^{-1}
Pb	10 - 200			37.97	2.5-28		3
Bi							3.0x 10^{-1}
Al	250 - 5000						
B	10000 - 40000					Max 80000 as boric acid	
Br	200 - 2500						
Co	2 - 50						
Cu	100 - 2000			500.57	73-346		
Fe	800 - 5000		3.7				
Mg	50 – 300 [a]		70		99-124 [a]		
Ni	10 - 200			55.15	3-28		
Na	5 – 30 [a]						
Zn	30 – 1000 [a]		0.3	433.95	140-1359		
Ti	40.4				70-186		
Sb		1.65					
Cs		4.00					
La		0.70					
Ce		1.36					
U		0.55			< 0.02-1.2		
K			488		899-1395[a]		
Na			36				
Ca			50		28-73		
Cr			0.8	255	3-61		
Ag				7.01	< 0.05-1		
Sn					< 0.07-1		
P							

STabla 1- 3. Efectos de los metales en la salud y la calidad del vino. A un pH gástrico, los iones libres de metales están disponibles para la absorción y puede causar efectos tóxicos. Su toxicidad se ha relacionado principalmente al estrés oxidativo y la interferencia con otros metales la mayoría esenciales.

Elemento	
Cu, Zn, Fe	Son requeridos en un amplio rango de procesos fisiológicos, y tienen algunos beneficios nutricionales. Su consumo excesivo conduce a síntomas gastrointestinales.
Cu	La exposición prolongada puede producir daño hepático y de riñones.
Al, As, Cd, Pb	Elementos tóxicos, sin valor nutricional conocida o rol fisiológico en el organismo humano.
Al	Se asocia a una serie de trastornos degenerativos (enfermedad de Parkinson y Alzheimer). Algunos ácidos orgánicos presentes en concentraciones apreciables pueden aumentar la absorción de Al en el tracto intestinal.
As	Carcinógeno para animales y humanos (piel, pulmón y vejiga). Híper o hipo pigmentación, queratosis, enfermedades cardiovasculares, disfunción de los riñones, diabetes.
Pb	Afecta el sistema nervioso y la biosíntesis de hemoglobina. Incluso a niveles muy bajos produce hipertensión, enfermedades cardiovasculares, disfunción en los riñones, deterioro de la síntesis ósea, disminución de la producción de esperma, osteoporosis.
Cd	Teratogénico, carcinogénico. Incita bioacumulación en el riñón e hígado. Ingesta prolongada produce lesiones celulares. Inhibe la acción de las enzimas de Zn y Se por sustitución.

***Fuente*:** (Waterhouse et al,. 2016)

2. Analytical testing of the interference standard method (IFS) for metals in wines by inductively coupled plasma mass spectrometry

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Abstract

An analytical studied of interference standard method (IFS) for metals in wines with low and high residual sugar by inductively coupled plasma mass spectrometry (ICP-MS) is evaluated. Based on theses major metals in wine, like Fe, Mn and Zn, the IFS method was an assessment. In this approach, an ion formed in the argon plasma, such as natural $^{38}Ar^+$ or as contaminant $^{83}Kr^+$ or also as an ion provided from the matrix sample (such as $^{13}C^+$), were utilized as IFS probe. The analytical signal was obtained from the ratio between the original signals of the analyte and these species IFS probe. The accuracy was confirmed using a *t*-test statistic for comparison between the concentrations obtained by FAAS and ICP-MS. In a first study, the IFS effect was analyzed by comparing the correlation coefficients obtained with or without dividing the analytical signal by the IFS. Better results in the determination of Fe, Mn and Zn were always obtained while using the $^{83}Kr^+$ IFS probe when compared to $^{13}C^+$ and $^{38}Ar^+$.

Keywords: IFS, isobaric interferences, mass spectrometry, wine analysis.

2.1. Introduction

The determination of the major, minor, trace and ultra-trace metallic elements in wines are important as those may have effects on the organoleptic properties and usually, the concentration levels are routinely monitored throughout all stages of wine production [1]. Normally wines from Europe, America, Africa or Asia, the major metallic elements found are Na, K, and Mg (at the mg L^{-1} levels) and the minor are heavy metals (as Fe, Cu, Zn, Cd, Pb, etc., all at the μg L^{-1} levels) [2,3]. Usually, these metallic elements are determined by flame atomic absorption spectrometry (principally alkalines, Fe, Mn, Zn, Cu, etc.) or graphite furnace atomic absorption spectrometry (principally Cd, Pb, As, Co, Ni, etc.). However, inductively-couple plasma mass spectrometry (ICP-MS) and inductively-couple plasma optical emission spectrometry (ICP-OES) are the techniques most extensively used to perform the elemental characterization of wines [4-6]. A critical evaluation and discussion of recent applications on atomic spectrometry methods for wines analysis have been reviewed [4]. Such works explores the multi-element capabilities of inductively coupled plasma–mass spectrometry with collision/reaction cell technology (CCT–ICP–MS) for the simultaneous determination of both spectrally interfered and non-interfered nuclides in wine samples using a single set of experimental conditions and pinpoint the main advantages and drawbacks associated to this kind of analysis, highlighting the effect of interferences linked to sample matrix as well as the strategies available to mitigate them through sample pretreatment, sample dilution, sample digestion, etc. One of the most common interfering organic compound in wines is ethanol and residual sugar, whose typical concentrations are respectively between 9 to 18% (v/v) and between 1 to 2% (p/v) [5] and can change the signal of some analytical signals (for example, As and Se) [7-10]. Currently, ICP-MS is one of the most appropriate techniques for the determination of trace elements in wine [11]. However, the formation of molecular species in the plasma, such as Ar^{2+} , ArO^+ , ArN^+ , ArH^+ , MAr^+, ArX^+ (M is a metal and X a nonmetal) and other polyatomic species (MO^+ , MOH^+ , XO^+ , XO^{2+} , XOH^+) that cause isobaric interferences may worsen precision and accuracy [12-14]. Additionally, the formation of oxides can also deteriorate the sensitivity for elements such as U, Ba and lanthanides [15,16].

In general, the capacity for determining at ultra-trace level elements is inherent to ICP-MS, but it is well known that low resolution equipments with quadrupole mass analyzers are affected by spectral interferences [17]. Nowadays these interferences may be resolved using collision and reaction devices for destroying and fltering ion-molecular species [4, 18]. Recently, a simple strategy based on the so-called interference standard method (IFS) has been proposed [19-23]. In this approach, an ion naturally formed in the argon plasma, such as Ar^+ or ArO^+, is monitored and the ratio between the signals of the analyte and these species is used to improve accuracy. Similarly, also the use the specie $^{129}Xe^+$ has been used as internal standard for the precise and accurate determination of several trace elements in different kinds of water samples by ICP-MS [24].

In this work, an analytical testing of the IFS method is evaluated and applied for wine with low and high residual sugar for three metallic elements (Fe, Mn and Zn) by ICP-MS. These elements were selected as model in the application of IFS, but this last approach also could be used for other elements as Cd, Pb, Hg, As, Co, Ni, etc. The accuracy was confrmed using a *t*-test statistic for comparison between the concentrations obtained by FAAS and ICP-MS by using as IFS probe $^{83}Kr^+$, $^{13}C^+$ or $^{38}Ar^+$.

2.2. Experimental

2.2.1. Reagents and materials

Aqueous solutions were prepared with analytical grade chemicals and deionized water (resistivity higher than 18.2 MΩ cm) obtained from a Milli-Q water purifcation system (Millipore, Bedford, MA, USA). Nitric acid and ethanol (both from Merck, Darmstadt, Germany) were separately distilled in quartz sub-boiling still (Milestone, Sorisole, Italy). Fe, Mn and Zn calibration solutions were prepared by dilution from a 1000 mg L^{-1} stock solution (Titrisol concentrate, Merck, Darmstadt, Germany). For the synthetic wine sample the reagents were: Sucrose as sugar, Potassium, Calcium, Copper, Acetic Acid, Tartaric Acid (all Merck, Darmstadt, Germany). All standard solutions were prepared using a polypropylene graduated tubes (Falcon®, 15 mL) which previously were kept in a 10% (v/v) chloride acid solution for 24 h and rinsed with ultra-pure water.

2.2.2. Apparatus

An inductively coupled plasma mass spectrometer (ICP-MS, Varian 820-MS, Mulgrave, Australia) with quadrupole mass analyzer was used in all experiments. The sample introduction system was composed of concentric nebulizer and double path spray chamber (Scott type) made with perfluoroacetate (PFA) and an automatic sampler (SPS3, Varian). An additional gas flow controller (AGM-1, Varian) for adding oxygen (99.99%, White Martins, Sertãozinho-SP, Brazil) as auxiliary gas in the ICP was employedTable 2- 1 presents instrumental and operating conditions. A flame atomic absorption spectrometry (Perkin Elmer model Analysis 300) equipped with a deuterium lamp background corrector.

The instrument parameters for determination of Fe, Mn and Zn were those recommended by the equipment manufacture [25]. **Table 2- 2** presents instrumental and operating conditions.

Table 2- 1. ICP-MS operating conditions

Operating conditions		
Plasma gas flow rate	(L min^{-1})	18
Auxiliary gas flow	(L min^{-1})	1.80
Nebulizer gas flow	(L min^{-1})	0.95
Sheath gas flow rate	(L min^{-1})	0.12
Peristaltic pump rate	(rpm)	7
Sampling depth	(mm)	5.5
RF power	(kW)	1.4
Points per Peak		3
Scan per replicate		3
Scan per sample		3
Dwell time	(ms)	1

Table 2- 2. FAAS operating conditions.

Instrumental parameters		Fe	Mn	Zn
Filament current	mA	30	20	15
Wavelength	nm	248.3	279.5	213.9
Oxidant flow (Air)	L min^{-1}	10.0	10.0	10.0
Fuel flow (Acetylene)	L min^{-1}	3.00	3.00	3.00
Slit	nm	0.2	0.2	0.7
Background correction		Yes	Yes	Yes

On the other hand, a conventional Carls Zeiss Refratometer (degrees Brix, 0 – 30 °Brix) was used to determine the high residual sugar.

2.2.3. Sample wine and synthetic wine sample

Different commercial wines with low and high residual sugar were provided by Viña de Neira (Guarilihue, Región del Bio Bio – Chile) and represent two types of grapes: red

Pinot Noir (low residual sugar, 0,6% w/v) and white Moscatel de Alejandria (high residual sugar, 14 % w/v), both from Chile. The ethanol concentration in wines is around 12% (v/v).

Wine samples were analyzed without any step of sample digestion. The analysis by ICP-MS a simple 1:10 v/v dilution with 0.14 mol L^{-1} HNO_3 was performed to reach an ethanol concentration around 1.2% (v/v). For FAAS analysis, direct introduction was used. Also to ensure that concentrations of metals in wine were correct, we made a cross-calibration, for which FAAS determinations and standard addition methods were performed in two different laboratories, the frst is the Instrumental Analysis at the Universidad de Concepcion (UdeC) and the second is the Environmental Chemistry at the Universidad Católica de la Santísima Concepcion (UCSC).

The above mentioned analyses were tested because it is necessary to provide reliability in the determination of Fe, Mn and Zn, which were subsequently compared with the determination accuracy with IFS and ICP-MS. In addition, this strategy was used to check the accuracy of the analytical determinations by ICP-MS because no available a certifed reference material wine. In general, all results obtaining from UdeC and UCSC showed not statically signifcant difference at a 95% confdence ($p>0.05$) [26].

In order to study the matrix effect in wine, a synthetic wine sample was prepared with ethanol 12 % v/v, residual sugar 0.6 % w/v, Fe^{3+} 0.96 mg L^{-1}, Mn^{2+} 1.4 mg L^{-1}, Zn^{2+} 0.40 mg L^{-1}, Cu^{2+} 0.15 mg L^{-1}, Tartaric Acid 1.2 g L^{-1}, Acetic Acid 0.50 g L^{-1}, K 980 mg L^{-1} and Ca^{2+} 74 mg L^{-1}. This concentration were employed because they are the levels normally found in wine.

2.3. Results and discussion

The effect of sample matrix in wine with low and high residual sugar represent an important limitation to the application of inductively coupled plasma mass spectrometry determinations. Isobaric polyatomic ions are normally critical on accuracy specially in situations where the analyte is dependent on the determination of a single natural isotope (i.e. represents 100% of the elements) or elements with more than one isotope (i.e where the abundant species not representing 100%), or when these major isotopes have a critical isobaric interference [20]. A case of critical isobaric interference is the determination by using ion isotope $^{56}Fe^+$ (abundance ^{56}Fe 91.66%) which is omnipresent with the interfering ion Ar overlap $^{40}Ar^{16}O^+$ and O overlap $^{40}Ca^{16}O^+$. In this case, is advisable to use the ion isotope $^{57}Fe^+$ even in low abundance (abundance ^{57}Fe 2.19%). In relation to Mn and Zn determinations, no isobaric interferences are critical (Mn: principal isotope specie $^{55}Mn^+$ (abundance ^{55}Mn 100%) in comparison with the interfering ion Ar overlap $^{40}Ar^{14}N^1H^+$ and Zn: principal isotope specie $^{64}Zn^+$ (abundance ^{64}Mn 48.89%) in comparison with the interfering ion O overlap $^{48}Ti^{16}O^+$). The use of IFS probe is not subject to a single species from Argon plasma (for example ion IFS probe as $^{38}Ar^+$ or from a contaminant as $^{83}Kr^+$). Thus, is feasible to use the ion isotope $^{13}C^+$ as IFS probe in wine samples because source of carbon (from ethanol and sugar) would supply a major natural abundance of ion $^{13}C^+$ (natural abundance of ^{13}C is close to 1.09%).

2.3.1. IFS strategy on repeatability and accuracy for Fe, Mn and Zn determination in wine

In relation to the critical isobaric interference of ion isotope $^{56}Fe^{+}$ by $^{40}Ar^{16}O^{+}$, in Fig. 2-1is showed the effect of wine matrix when is used a sample wine and synthetic wine sample (both Fe 0.96 mg L^{-1}) on repeatability (n=5) and accuracy when is recorded the original signal (Fig. 2-1a., isotope ^{56}Fe counts: for a wine sample and Fe standard) and the signal corrected by $^{83}Kr^{+}$ as IFS probe (Fig. 2-1b. by using IFS). In these conditions, when the original signal for wines is compared with the synthetic wine, a good repeatability is obtained (both analysis C.V. between 0.5 to 1.0%), however, the calculated accuracy is poor (both samples, statistic parameter $p < 0.001$, indicating statically signifcant difference at a 95% confdence). On the other hand, in comparison with using $^{83}Kr^{+}$ IFS as probe, a good accuracy is obtained (parameter $p = 0.364$), however a low repeatability was observed (both analysis between C.V. between 6 to 8%). Regarding this latter, and in relation to Mn and Zn analysis in wine, the normal loss of repeatability and the gain in accuracy, also were observed, and further, these analyses are also consistent with those reported in previous works [19-21].

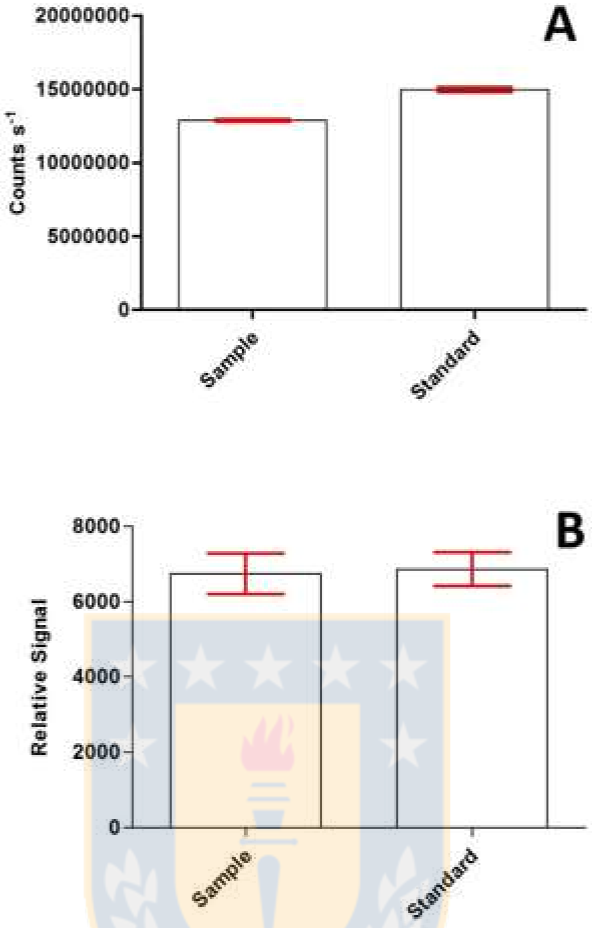

Fig. 2-1. Signal intensities for one wine sample (red pinot noir wine) and synthetic wine sample (both Fe 0.96 mg L^{-1} and replicas n=5). A). Original signal (counts) and b) analytical signal by using $^{83}kr^{+}$ IFS probe.

Since, the results show that used of IFS compromises the sensitivity for species $^{56}Fe^{+}$, $^{57}Fe^{+}$, $^{55}Mn^{+}$ and $^{64}Zn^{+}$ determination by ICP-MS leading to poor detection limits respect when is used the original signal. As example, the Fig. 2- 2 is presented the calibration curve for the ion isotope $^{55}Mn^{+}$ (original signal) and by using IFS probe as: $^{13}C^{+}$, $^{38}Ar^{+}$ and $^{83}Kr^{+}$. Based on slope of linear regression equation a signifcative decreasing is always observed when any IFS is used (*i.e.* $^{13}C^{+}$, $^{38}Ar^{+}$ or $^{83}Kr^{+}$). In this condition, the best analytical signal (ratio between original signal/IFS interference) is by using the $^{83}Kr^{+}$ IFS probe. In addition, based on correlation coefficient, also the best calibration curves are always obtained with this IFS probe. Regarding other metals (*i.e.* for ion based on ion isotope $^{56}Fe^{+}$ and $^{64}Zn^{+}$), some similar trends were also observed. Table 2- 3 shows a summary for limits of detection (LOD) and limits of quantification (LOQ) with and without IFS probes. The LOD and LOQ for all metals were calculated in according to report a previous work [20].

Table 2- 3. Limits of detection (LOD) and quantification (LOQ) achieved for Fe, Mn and Zn by using different IFS probe ($^{13}C^{+}$, $^{38}Ar^{+}$ and $^{83}Kr^{+}$).

Isotope	IFS condition	LOQ, µg L^{-1}	LOQ, µg L^{-1}
^{56}Fe	$^{56}Fe^{+}$	10	28
	$^{56}Fe^{+}/^{13}C^{+}$	120	333
	$^{56}Fe^{+}/^{38}Ar^{+}$	97	311
	$^{56}Fe^{+}/^{83}Kr^{+}$	28	55
^{55}Mn	$^{55}Mn^{+}$	5	14
	$^{55}Mn^{+}/^{13}C^{+}$	98	298
	$^{55}Mn^{+}/^{38}Ar^{+}$	34	76
	$^{55}Mn^{+}/^{83}Kr^{+}$	23	60
^{64}Zn	$^{64}Zn^{+}$	8	22
	$^{64}Zn^{+}/^{13}C^{+}$	160	424
	$^{64}Zn^{+}/^{38}Ar^{+}$	95	276
	$^{64}Zn^{+}/^{83}Kr^{+}$	17	54

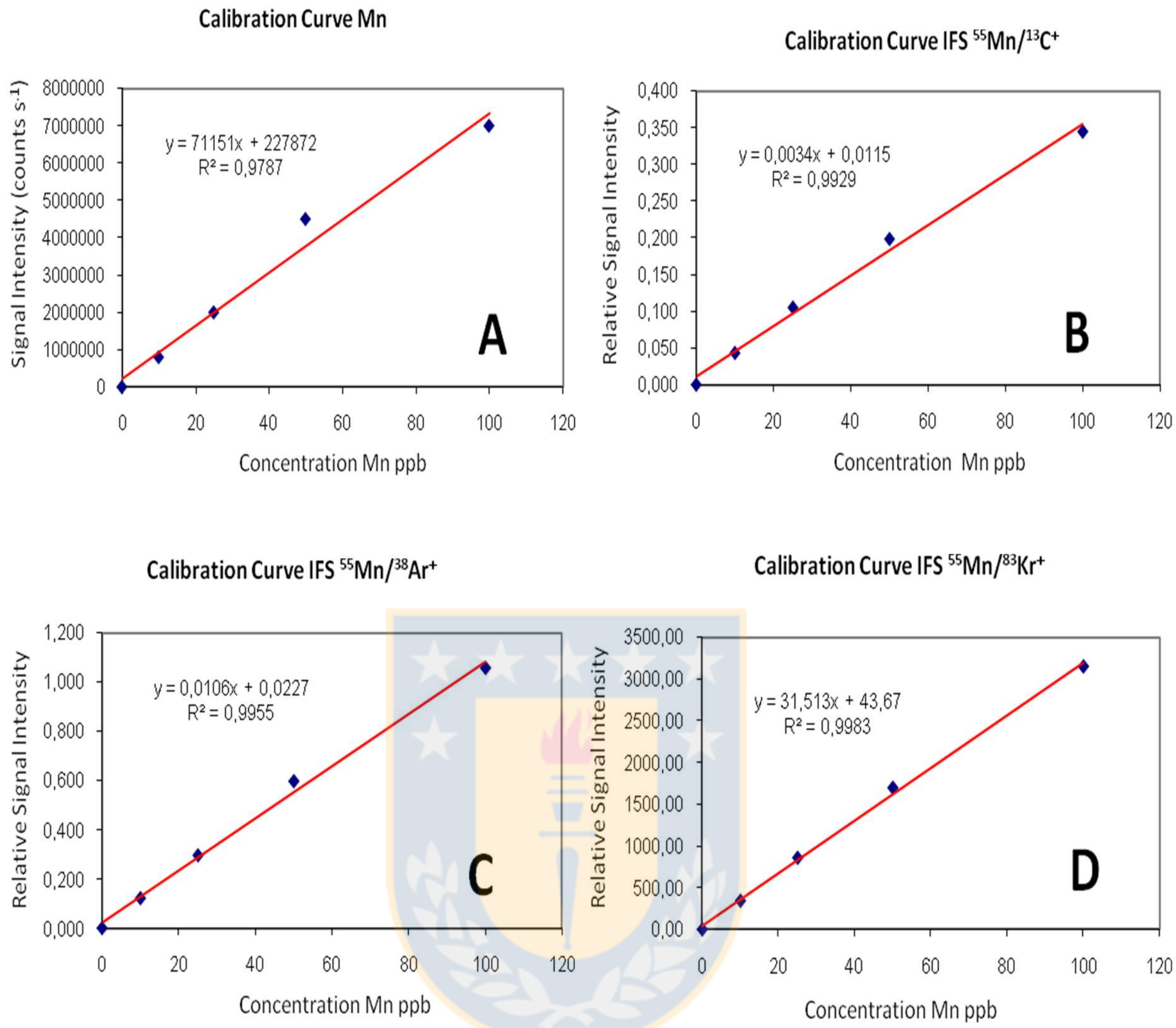

Fig. 2- 2. Calibration curves for Mn determination by using different IFS probes. A) Original signal; b) $^{13}C^{+}$ IFS probe; c) 38Ar^{+} ISF probe; and d) ^{83}Kr^{+} ISF probe

Table 2- 4 is presented the comparative study of the Fe, Mn and Zn determination obtained by using the original signal achieved by ICP-MS in comparison with those by FAAS (value accepted as true). This comparative study was performance in order the confrm the accuracy based a *t*-test statistic (parameter "p" to know if there is statistically signifcant differences). In this condition, a critical loss of accuracy by ICP-MS is observed for Fe and

Mn determination in wine with low residual sugar (all comparative determination $p < 0.05$). This loss of accuracy it is in relation to those isobaric interference mentioned above which probably is supply by wine sample matrix (for example critical isobaric interference: for isotope ^{56}Fe (91.72%): Ar overlap $^{40}Ar^{16}O^+$ and O overlap $^{40}Ca^{16}O^+$; for ^{57}Fe (2.2%): Ar overlap $^{40}Ar^{16}OH^+$ and O overlap $^{40}Ca^{16}OH^+$ and for ^{55}Mn (100%): Ar overlap $^{40}Ar^{14}NH^+$ and O overlap $^{39}K^{16}O^+$). Respect of Mn determination based on ion isotope $^{55}Mn^+$ the critical loss accuracy by the ion species $^{39}K^{16}O^+$ it is about the high concentration of potassium found normally in this wine ($K > 900$ mg L^{-1}). Regarding Zn determination, no critical isobaric interference is observed by the principal interference on ICP-MS determination based on ^{64}Zn (48.6%) (titanium overlap $^{48}Ti^{16}O^+$), because probably the supply of titanium concentration from this wine is low. Conversely, respect Zn determination based on ion isotope ^{66}Zn (27.9%), which has been not a critical isobaric interference, a loss accuracy is observed ($p < 0.05$). In relation the last, we have no justification for this not expected a result.

Table 2- 4. Statistical comparison by using the original signal for Fe, Mn and Zn determinations in wines with low residual sugar by ICP-MS and FAAS. Red pinot noir wine with residual sugar 0.6% w/v.

	By ICP-MS		**By FAAS**	
Isotope	**Linear equation**	**Fe concentration found* mg L^{-1}**	**Fe concentration found* mg L^{-1}**	**p****
^{56}Fe	Y=32307X+2633 R^2=0.9986	3.97 ± 0.01	0.961 ± 0.020	0
^{57}Fe	Y=1624.4X-91.8 R^2=0.9979	1.97 ± 0.04	0.961 ± 0.020	0
^{55}Mn	Y=68762X+77162 R^2=0.9950	2.041 ± 0.05	2.18 ± 0.02	0.01
^{64}Zn	Y=8180.1X+844.8 R^2=0.9993	0.267 ± 0.017	0.284 ± 0.003	0.163
^{64}Zn	Y=4937.5X+009.6 R^2=0.9996	0.378 ± 0.026	0.284 ± 0.003	0.003

* The values reported are the average, and ± is the mean standard deviation, *n* = 3. ** Statistical parameter, $p > 0.05$ indicates no statistically significant difference at 95% confidence.

Tables 2 - 5 and Tables 2 – 6 show the comparative results (accuracy) regarding the use of ^{83}Kr^{+} IFS probe in Fe, Mn and Zn determinations by ICP-MS in comparison with those obtained by FAAS in wines with low and high residual sugar, respectively. The accuracy was newly confirmed using a *t*-test statistic for comparison between the concentrations obtained by FAAS and ICP-MS. Based on the best result of the statistical parameter "p," no statistically significant differences (always statistic parameter $p > 0,05$) were observed between values obtained by FAAS and ICP-MS using the ^{83}Kr^{+} IFS probe for ^{56}Fe^{+}, ^{57}Fe^{+}, ^{55}Mn^{+}, ^{64}Zn^{+} and ^{66}Zn^{+} in wine with low or high residual sugar. Respect this last results it is consistent with previous work for the determination of sulfur, Mn, and Fe in foods [21] and also for sulfur in biodiesel [22]

Table 2- 5. Statistical comparison by using ^{83}Kr$^+$ IFS probe for Fe, Mn and Zn determinations in wines with low residual sugar by ICP-MS and FAAS. Red pinot noir wine with residual sugar 0.6% w/v.

Isotope	**By ICP-MS IFS ^{83}Kr$^+$**		**By FAAS**	**p****
	Linear equation	**Fe concentration found* mg L^{-1}**	**Fe concentration found* mg L^{-1}**	
^{56}Fe	Y=20.23X+8.071 R^2=0.9961	0.896 ± 0.073	0.961 ± 0.020	0,21
^{57}Fe	Y=0.8172X-0.5629 R^2=0.9970	1.23 ± 0.09	0.961 ± 0.020	0.01
^{55}Mn	Y=31.513X+4.67 R^2=0.9983	2.31 ± 0.10	2.18 ± 0.02	0.09
^{64}Zn	Y=3.7532X+0.03585 R^2=0.9998	0.306 ± 0.025	0.284 ± 0.003	0.20
^{66}Zn	Y=2.2652X+0.02355 R^2=0.9997	0.427 ± 0.038	0.284 ± 0.003	0.003

* The values reported are the average and ± is the mean standard deviation, *n* = 3.

** Statistical parameter, $p > 0.05$ indicates no statistically significant difference at 95% confidence.

Table 2- 6. Statistical comparison by using ^{83}Kr$^+$ IFS probe for Fe, Mn and Zn determinations in wines with high residual sugar by ICP-MS and FAAS. White Muscat of Alexandría wine with residual sugar 14% w/v.

Isotope	**By ICP-MS IFS ^{83}Kr$^+$**		**By FAAS**	**p****
	Linear equation	**Fe concentration found* mg L^{-1}**	**Fe concentration found* mg L^{-1}**	
^{56}Fe	Y=20.23X+408.071 R^2=0.9961	0.879 ± 0.286	1.02 ± 0.02	0.47
^{57}Fe	Y=0.8172X-0.05629 R^2=0.9970	1.50 ± 0.32	1.02 ± 0.02	0.05
^{55}Mn	Y=31.513X+3.67 R^2=0.9983	1.40 ± 0.19	1.45 ± 0.01	0.70
^{64}Zn	Y=3.7532X+0.3585 R^2=0.9998	0.260 ± 0.038	0.294 ± 0.003	0.20
^{66}Zn	Y=2.2652X+0.2355 R^2=0.9997	0.379 ± 0.056	0.294 ± 0.003	0.06

* The values reported are the average and ± is the mean standard deviation, *n* = 3.

** Statistical parameter, $p > 0.05$ indicates no statistically significant difference at 95% confidence.

As possible approach regarding the application of different IFS probe, we consider ion isotope $^{13}C^+$ as IFS testing probe in a similar manner to $^{56}Fe^+$, $^{55}Mn^+$, $^{64}Zn^+$ and $^{66}Zn^+$ determination in wine with high and low residual sugar. As has been mentioned above, this study was taken into account because wines with high residual sugar supply for $^{13}C^+$. This result is show in Table 2 - 7. The best results are obtained for Fe y Zn determination ($p>0.05$) when the ion isotopes $^{56}Fe^+$ or $^{57}Fe^+$ or $^{64}Zn^+$ are used. In consideration of the use of the ion isotope $^{57}Fe^+$ (with a low natural abundance, 2.2%) the use of $^{13}C^+$ in these conditions would able for Fe analytical determination, however, a low sensitivity is currently obtained for the three elements studied (Table 2- **7**, see slope of the calibration curve) when compared respect the used $^{83}Kr^+$ or $^{38}Ar^+$ as IFS probes (Table 2- 5 and Table 2- 6).

Table 2- 7. Statistical comparison by using $^{13}C^+$ IFS probe for Fe, Mn and Zn determinations in wines with high residual sugar by ICP-MS and FAAS. White Muscat of Alexandría wine with residual sugar 14% w/v.

	By ICP-MS IFS $^{13}C^+$		**By FAAS**	
Isotope	**Linear equation**	**Fe concentration found* mg L^{-1}**	**Fe concentration found* mg L^{-1}**	**p****
^{56}Fe	$Y=0.0013X+0.00057$ $R^2=0.9837$	0.221 ± 0.572	1.02 ± 0.02	0.08
^{57}Fe	$Y=8x10^{-5}X+4x10^{-6}$ $R^2=0.9972$	1.04 ± 0.13	1.02 ± 0.02	0.71
^{55}Mn	$Y=0.0034X+0.00115$ $R^2=0.9929$	1.14 ± 0.08	1.45 ± 0.01	0.01
^{64}Zn	$Y=0.0004X+0.00008$ $R^2=0.9987$	0.300 ± 0.020	0.294 ± 0.003	0.63
^{66}Zn	$Y=0.0002X+0.00005$ $R^2=0.9989$	0.346 ± 0.022	0.294 ± 0.003	0.02

* The values reported are the average and ± is the mean standard deviation, *n* = 3.
** Statistical parameter, $p> 0.05$ indicates no statistically significant difference at 95% confidence.

2.4. Conclusions

The IFS method using $^{38}Ar^+$, $^{83}Kr^+$ or $^{13}C^+$ as a probe in ICP-MS determinations is an effective alternative to reducing isobaric interference in wine sample. The best accuracy for Fe, Mn and Zn determinations in wines with high and low residual sugar is by using $^{83}Kr^+$ as IFS probe. It was the first time that the IFS method was applied for major metals in wine in a complex matrix sample and it supports the idea that this strategy can be requested for other elements, for example for theses in low concentration (Cd, Pb, Hg, As, Co, Ni, etc.), or also can be widely applied in other types of matrices, as oils, brines, seawater, biological samples, etc. More studies are being carried out and may provide additional information, which could support successful implementation of the IFS method.

2.5. Acknowledgments

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3. Geographical origin classification by elemental composition of monovarietal and young Chilean wines

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Abstract

The elemental composition of monovarietal and young wines from different winegrowing valleys in Chile was combined with chemometric treatment to predict the geographical origin. A total of 183 samples of both white and red wine were collected from important vineyards into 2 regions and from four important subareas o valleys: in Central region; Rapel, Curico and Maule valleys and south region; Itata valley. The macro and micro elements were determined by Flame Atomic Absorption Spectrometry (FAAS) and Flame Atomic Emission Spectrometry (FAES). Elements in trace levels as such as As, Pb, Tl, and U were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The elemental patterns cannot be attributed to a specific area, and all elements were used to prediction models. Soft Independent Modeling of Class Analogy and Linear Discriminant Analysis model showed accurate predictions according to the four valleys that in 84 and 90 percent of the cases were correctly predicted. The inorganic composition can be used as a discrimination model for geographical origin differentiation of young wines from these Chile's valleys.

Keywords: wines, Chile's valleys, inorganic composition, geographic differentiation, chemometric.

3.1. Introduction

Chile is well known for the good geography and climate for wines production, which arouse the attention of consumers to know more about the wines characteristic, quality, and origin [1,2]. It also has noteworthy numbers of vineyards distributed throughout the territory that contribute to exports, the domestic market of wine consumed and the bulk wine [3].

These vineyards are on subregions or valleys, and perhaps some of the significant aspects of these are the geography and climate [1, 2]. It has been recognized that the geographic origin of the product is an attribute that is particularly useful to new wineries that have not developed a strong brand image of their own. Research about patterns of geographical origin differentiation has been carried out, due to the importance of establishing parameters as methods of control, quality, and food safety [4]. The Chilean zones of wine growing are usually close to the Andes Mountains and near the Pacific Ocean (among 180 km wide on average) and from north to south (extending for 2.300 km), following the course of tectonic depressions filled by river flooding.

The elemental profile of wines is associated with composition and properties of soils as well as by absorption processes of the plants and bioavailability, denominated endogenous factors, as well as influenced by exogenous factors such as viticulture practices (e.g., application of pesticides, irrigation) and the environment pollution [5,6].

Several studies have been carried out worldwide stating that the elements present high potential as fingerprint [7–12]. Thiel et al., determined 19 elements by ICP-MS in white wines of wine growing areas from Germany. Arsenic, Be, Co, Cs, Ga, Li, Nb, Ni, Rb, Te, Ti, W, Y, Zr, Mo, Cd, Sb, Tl, U and rare earths elements (REE) were defined as the most discriminant elements in the determination of geographical origin generating 88.6% of correct prediction [13]. On the other hand, Coetzee et al., defined the basic patterns for wines from South Africa in both white and red ones, by using ICP-MS technique, Aluminum, Mn, Rb, Ba, W, Tl, Y, U and Cr concentrations were the important variables for 100% of the correct classification by means of cross-validation [11]. In wines from wine-producing countries in South America (Argentina, Brazil, Chile, and Uruguay), the concentrations of 45 elements in 53 red wines were determined, and Tl, U, Li, Rb, and Mg were the discriminant elements [14]. Fabani and coworker, classified wines and soil from three production areas of Argentina, eleven elements were determined in 31 wine samples. The principal descriptors of LDA and the highest F-value were K, Fe, Ca, Cr, Mg, Zn, and Mn, allowing classify correct classification of 100% of the wines analyzed from the three regions [15]. Azcarate et al. analyzed 57 white wine samples of the main varieties from four winegrowing regions of Argentina, the results of principal component analysis explained 95.95% of the variance data total and five ultratrace elements (Ba, As, Pb, Mo, and Co). The rates of discrimination in similar works are higher than 96% for prediction and validation [12]. The most usual element for wine authentication in general are Ca, Mg, Mn, Rb, Sr, Zn, Pb, Li among others [5,16].

In Chile, related studies with characterization of wines among producing regions were focused on varietals. Works such as that by Villagra et al., based their study on the varietal discrimination of red and white wines from the Elqui valley and Itata valleys [17]. The varieties Chardonnay, Sauvignon blanc, Cabernet Sauvignon, Carménère, Pinot noir and Syrah were determined by mass spectrometry with Fourier transform (ESI FT-MS). The model validation showed correct classification of 76% (white) and 62% (red). Saavedra et al., studied pinot noir wines from Casablanca valley and the classification of the subareas from non-commercial products and their metallic profiles (ICP OES), with 77.50% of classification [18]. Finally, V.F. Laurie et al., carried out an important investigation by PCA based on a set of 75 wine samples of commercial wines obtained directly from wineries or purchased from local supermarkets or wine stores, from Elqui, Limarí, Casablanca, Maipo, Colchagua, Maule and Itata valleys in Chile [19]. The Na concentration was evidenced in the samples analyzed with flame AAS (FAAS) as a distinctive pattern. In general, some of the most common problems related to the development of these research are those associated with the degree of inclusion of anthropogenic sources (environmental pollution) and exogenous factors (climatic changes, agricultural practices, and winemaking processes), as well as the contribution of the endogenous factors [5]. However, there is not an adequate methodology for the classification of wine-producers regions based on the elemental composition of Chilean wines.

Atomic Absorption Spectroscopy (AAS) [7,20], Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) [21], and inductively coupled plasma-mass spectrometry (ICP-MS) [15,16,22,23], are main techniques employed for inorganic determination

Chemometric tools to classify and predict from patterns of chemical elements distribution are usually principal PCA component analysis, a non-supervised technique based on linear combinations of weighted variables. It has the capability of reflecting the natural clustering of the samples and the studied variables in a multidimensional spectrum, without forcing the dataset and variables to be organized due to the similarities among them. With the research of PCA explained by the three first principal components (PCs), it is possible to explain the percentage of cumulative variance because it is condensed information of the matrix and usually expected of the total variability [23,24]. Linear Discriminant Analysis (LDA) aims to dividing the sampling space into subspaces using hyperplanes that allow performing a better separation of the study groups [25]. On the other hand, soft independent modeling of class analogies SIMCA consists of a collection of n PCA models, one for each of n defined classes, LDA. SIMCA is a supervised pattern recognition technique and provides statistical models with 95% confidence for results on both classification and prediction, in a range between 80.8%–100% and 74%–100% [6,13,14,26].

The term “young monovarietal wine” relates to a wine made with minimum chemical and technological intervention in growing grapes, and in winery practices when preparing the product. Thus, when exogenous factors are minimized, it is possible to improve the original classification of wines based only on endogenous factors.

The main goal of this study was to develop and validate the relation between the geographical origins of young monovarietal wine and inorganic composition by chemometric tools. The selected wine samples are from four major producing valleys of Chile (Rapel, Curicó, Maule, and Itata) and the study was based on the inorganic composition of the following elements: Na, K, Ca, Mg, Fe, Zn, Mn, Rb, Li, Sr, As, Tl, Pb, and U. The determination of the elements was carried out by FAAS, FAES and ICP-MS.

3.2. Materials and methods

3.2.1. Study area and wine samples

183 wine samples, 36 samples of white wine and 147 red wines were collected from four major wine production areas of Chile: Rapel, Curicó, Maule and Itata valleys (Fig. 3-1). Wine samples were acquired directly in vineyards located in these areas.

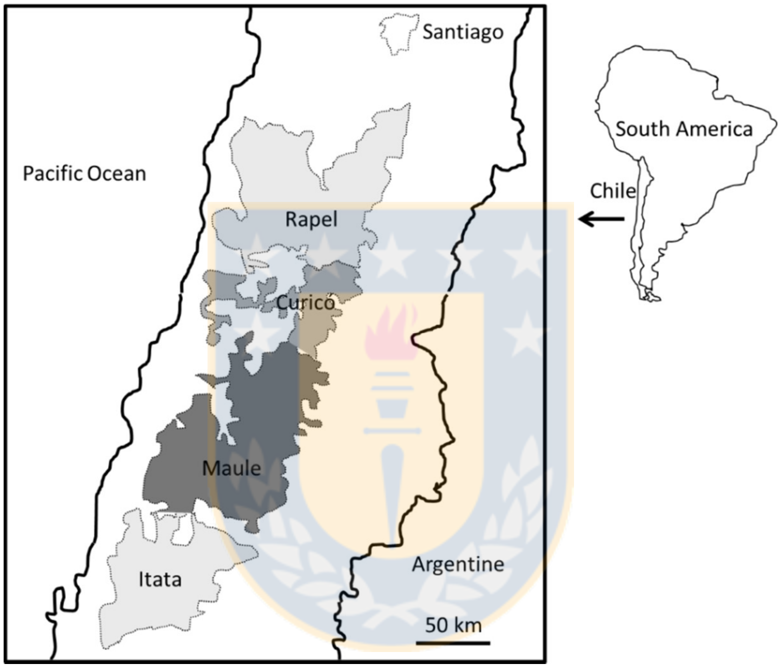


Fig. 3- 1. The geographic area of valleys in Chile used in our study: Rapel. Curicó. Maule and Itata

The white wines were assembled after the finished alcoholic fermentation and the red wines after the completed processes of malolactic fermentation; all samples were monovarietal and young wines (Table 3- 1). The procedure described has focused on a reduction of exogenous factors facilitating a better degree of reliability in the differentiation of origin of Chilean wines.

3.2.2. Reagents

Standard solutions used for the calibration curves set were prepared by dilution of stock analytical standards 1000 mg L^{-1} Fe, Mn, Zn, Li, Rb, Sr, Mg, Ca, K and Na (Merck, Darmstadt, Germany). Ethanol 98.8% v/v (Merck, Darmstadt, Germany) was used for the matrix-matched calibration curve. All solutions were prepared using deionized water (resistivity higher than 18.2 MΩ cm) obtained from a Milli-Q water purification system. Hydrochloric acid (Merck, Darmstadt, Germany) 25% v/v was used in Zn, Fe, Mn, Mg and Sr measurements and La (Merck, Darmstadt, Germany) 5% v/v in Ca determination.

Table 3- 1. Identification of wine samples

	Rapel (n=34)	**Curico (n=22)**	**Maule (n=29)**	**Itata (n=62)**
Red	Cabernet Sauvignon (*n*=10)	Cabernet Sauvignon (*n*= 6)	Cabernet Sauvignon (*n*=9)	Cabernet Sauvignon (*n*=8)
	Carménère (*n*=10)	Carménère (*n*=4)	Carménère (*n*=6)	Carménère (*n*=4)
	Merlot (*n*=10)	Merlot (*n*=3)	Merlot (*n*=3)	Merlot (*n*=3)
	Syrah (*n*=5)	Syrah (*n*=2)	Syrah (*n*=2)	Syrah (*n*=2)
	Pinot noir (n=2)	Pinot noir (*n*=2)	País (*n*=5)	Pinot noir (*n*=5)
	Others (*n*=1)	Malbec (*n*=3)	Other (*n*=6)	País (*n*=12)
		Others (*n*=2)		Cinsault (*n*=16)
				Other (*n*=14)
	Rapel (*n*=4)	**Curico (*n*=7)**	**Maule (*n*=1)**	**Itata (*n*=24)**
White	Chardonnay (*n*=2)	Chardonnay (*n*=2)	Muscat torontel	Muscat of Alexandria (*n*= 13)
	Viogner (*n*=1)	Sauvignon blanc (*n*= 3)		Muscat sparkling (*n*=4)
	Sauvignon blanc (*n*=1)	Others (*n*=2)		Late harvest Muscat (*n*=4)
				Chardonnay (*n*=1)
				Sauvignon blanc (*n*=2)

3.2.3. Instrumentations

A Perkin-Elmer Model AAnalyst 300 flame atomic absorption spectrometer equipment was optimized for Ca, K, Fe, Mn, Mg, and Zn determination. The same instrument was used in emission mode for determination of Rb, Li, Na and Sr. Analyses were carried out according to the guidelines of the Compendium of International Methods of Wine and Must Analysis (OIV, O. 2009). As, Pb, Tl, and U determination was carried out using ICP-MS/MS (Agilent 8800, Tokyo, Japan) in ICP-MS mode. All operating parameters are described in STable 3- 1.

3.2.4. Analytical procedure

Samples were determined directly by FAAS avoiding digestion steps and the standard solutions. All determinations were analyzed in triplicate. To avoid interference of phosphates in the flame, standard solutions of Zn, Fe, Mn, Mg and Sr in HCl 1% v/v were prepared. The Ca standard were prepared with 50 µL of La^{3+} at 0.2% v/v. The determination of As, Pb, Tl, and U was carried out by diluting wine samples in deionized water 1:10 v/v (wine: water) containing HNO_3 1% v/v. Also, the linear equation was performed by using the linear regression method. The relative standard deviation (RSD) and limits of detection (LOD) and quantification (LOQ) were also calculated according to IUPAC recommendations as 3 and 10 times the standard deviation (n = 10) of blank (1.2% v/v of ethanol), respectively. The analysis of the reference material for enology laboratory was performed to establish the accuracy of the procedure within the 95% confidence interval for the determination of K, Fe and Ca. *P* values were calculated and compared to the treatment sample developed (see STable 3- 5).

3.2.5. Multivariate analysis

For an appropriate treatment of data, the relations between elements and valleys were identified. The chemometric techniques used in this study were an analysis of variance (ANOVA), PCA, SIMCA, and LDA. For multivariate data analysis, the statistical packages Infometrix Inc, with the software Pirouette ver. 4.0, Statgraphics Centurion, version 16.1 (2010) and XLSTAT version 1 (2016) were used.

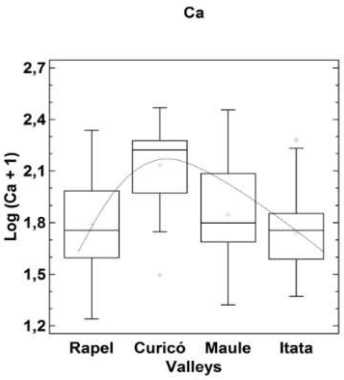
3.3. Results and discussions

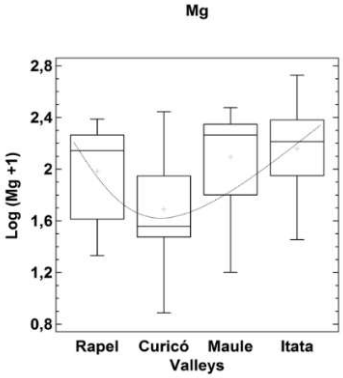
3.3.1. The content of the element in the red and white wines

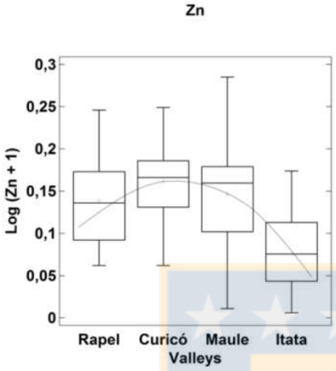
White and red wine samples included 183 samples grouped into four classes (Curicó, Rapel, Maule and Itata valley) and related to fourteen variables: Na, K, Ca, Mg, Fe, Zn, Mn, Rb, Li, Sr, As, Tl, Pb, and U. The results for elemental concentrations, limit of quantifications and detection and deviations standards are resumed in STABLE 3- 2. The logarithm of the concentration of the element plus one [log (variable concentration) + 1] were transformed to a group of normalized data. Concentration range means and standard deviations values of elements found in young wines are resumed in Table 3- 2**.** The variables exploration by box plots is represented in Fig. 3- 2**.** Recoveries between 83 to 125% on average 104 ± 10 were calculated for the other elements not included in the reference material, the results for these are in STable 3- 5.

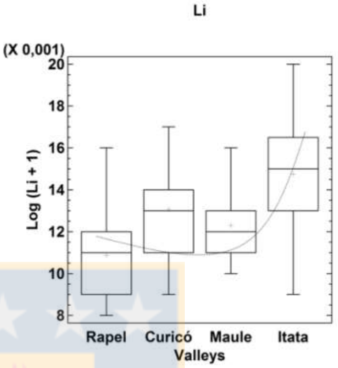
The results of major elements concentrations were found in normal ranges according to the results of V.F. Laurie and Bentlin [14,19]. The ANOVA, 95% confidence level, $p<0.05$, showed the significant difference to Zn, Li, Ca, Rb and Mg in the four valleys according to *F* -reason, 9.43, 7.28 and 5.59, 4.87, 3.58 (p = 0) respectively. The ranking with potentials to differentiate all wines was elaborated: Zn> Li> Ca> Rb = Mg> As = K> Mn = Tl = Pb> Fe> Sr> U. The results of ANOVA show from the four studied areas the decrease behavior in the concentration of Ca, Fe, K, Zn, Mn, Rb, Sr, Tl, As and U in geographical order from north to south. Whereas Mg, Li, and Pb in the Itata valley were significantly higher among the group of elements to this valley and among the valleys.

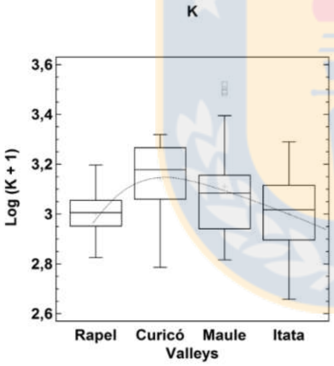
Otherwise, the content of elements in red wines and white wines were investigated (Fig. 3- 3). In relation to red samples from Rapel valley are the middling values of concentrations. In Curicó, they contain the high quantity of K, Ca, Zn, Fe and Sr, as well as lower concentrations of Na and Mg. The Maule red samples showed the highest levels of Mn and Tl, but the lowest level of Li. From Itata valley, the elements have the inverse relation with Curicó elements, for K, Ca, Mn, Fe, Zn, and Sr, who showed the lowest levels. Besides, in this valley, the high concentrations of Na, Mg, Li, U, and Pb were established.

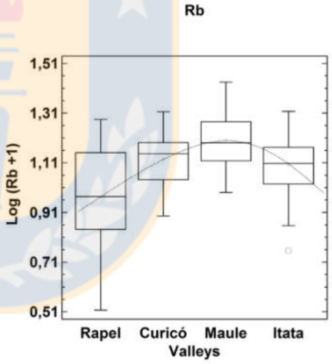

Ca
2,7
2,4
2,1
1,8
1,5
1,2
Log (Ca + 1)
Rapel
Curicó
Maule
Itata
Valleys

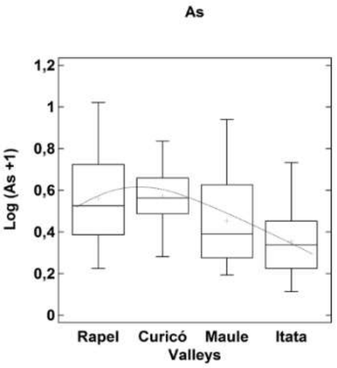

Mg
2,8
2,4
2
1,6
1,2
0,8
Log (Mg +1)
Rapel
Curicó
Maule
Itata
Valleys

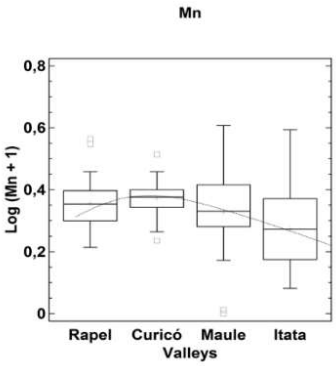

Zn
0,3
0,25
0,2
0,15
0,1
0,05
0
Log (Zn + 1)
Rapel
Curicó
Maule
Itata
Valleys


Li
(X 0,001)
20
18
16
14
12
10
8
Log (Li + 1)
Rapel
Curicó
Maule
Itata
Valleys


K
3,6
3,4
3,2
3
2,8
2,6
Log (K + 1)
Rapel
Curicó
Maule
Itata
Valleys


Rb
1,51
1,31
1,11
0,91
0,71
0,51
Log (Rb +1)
Rapel
Curicó
Maule
Itata
Valleys


As
1,2
1
0,8
0,6
0,4
0,2
0
Log (As +1)
Rapel
Curicó
Maule
Itata
Valleys


Mn
0,8
0,6
0,4
0,2
0
Log (Mn + 1)
Rapel
Curicó
Maule
Itata
Valleys

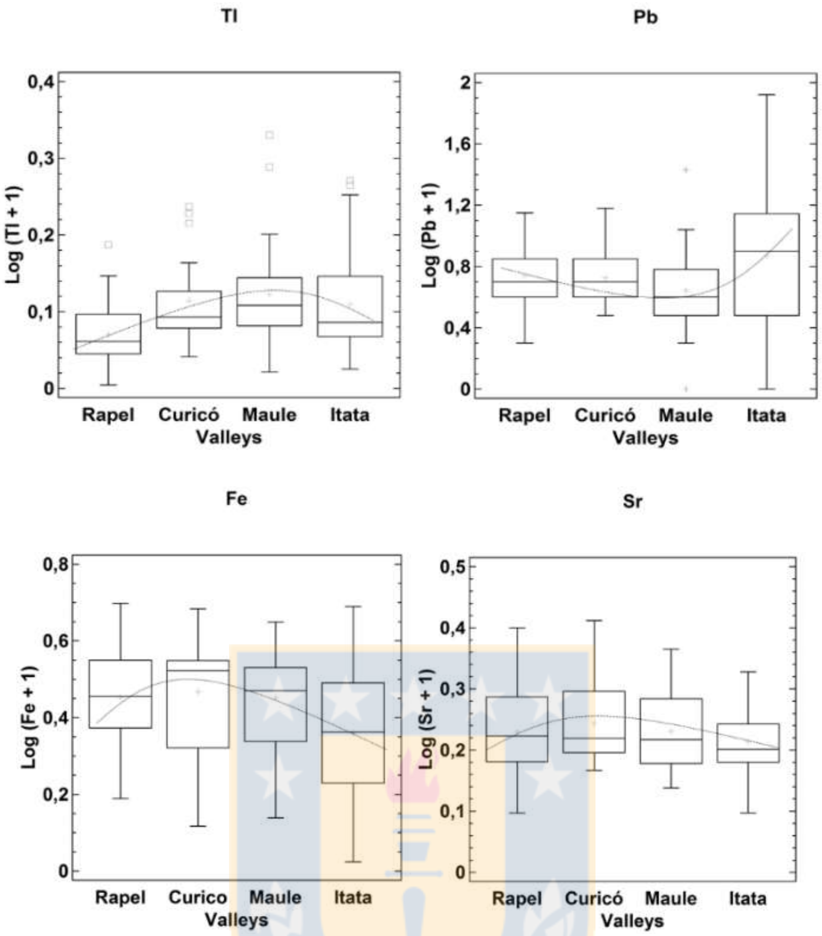


Fig. 3- 2. Box plots are showing the median and concentration range of Zn, Li, Ca, Mg and K.

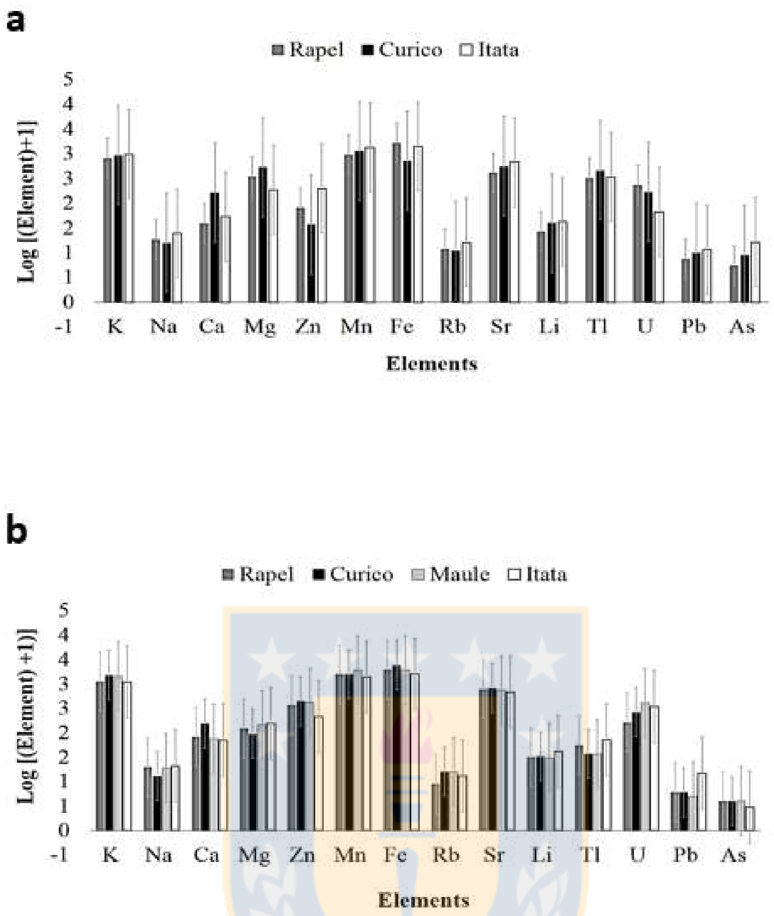


Fig. 3- 3. Mean concentration of elements for a. Red wines and b. White wines.

Table 3- 2. Concentration range. Means (in bold) and standard deviations (values in parenthesis) of minor and trace elements found in young red wines and white wines from Rapel. Curico. Maule and Itata.

	Red wines (n=147)				**White wines (n=36)**		
	Rapel (n = 34)	**Curico (n = 22)**	**Maule (n = 29)**	**Itata (n = 62)**	**Rapel (n = 4)**	**Curico (n = 7)**	**Itata (n = 24)**
K*	667 - 2840 **1115**(444)	384 - 2083 **1512**(467)	655 - 3320 **1449** (776)	454 - 1948 **1089** (360)	667 – 1069 **812** (263)	610 - 1176 **932** (285)	535 – 1783 **979** (301)
Na*	6 – 53 **19** (11)	1 - 24 **12** (6)	4 - 59 **18** (16)	2 - 93 **20** (21)	11 - 23 **18** (5)	8 - 21 **15** (6)	6 - 66 **23** (21)
Ca*	16 - 216 **81** (61)	30 - 293 **152** (70)	20 - 167 **75** (42)	23 - 284 **70** (47)	16 - 57 **39** (17)	93 - 264 **163** (76)	23 – 99 **52** (22)
Mg*	20 - 244 **120** (78)	22 - 278 **94** (94)	15 – 299 **145** (92)	27 - 437 **153** (91)	24 - 139 **81** (48)	7 - 88 **36** (27)	37 – 531 **186** (136)
Zn**	153 - 760 **372** (153)	152 - 774 **438** (438)	26 - 930 414 (205)	14 - 715 **212** (145)	199 - 556 **409** (151)	477 - 713 **556** (107)	15 – 378 **195** (114)
Mn**	790 – 4380 **1587** (776)	723 - 2611 **1563** (1563)	592 - 11762 **1902** (2022)	207 - 6630 **1385** (1344)	637 - 1271 **956** (288)	633 - 2037 **1131** (478)	207 – 4854 **1338** (1136)
Fe**	545 - 3994 1**954** (894)	487 - 3814 **2423** (2423)	378 - 3460 **1894** (786)	58 – 3673 **1576** (976)	878 - 2173 **1648** (623)	308 - 1096 **721** (333)	58 - 3895 **1398** (1066)
Rb*	2 - 18 **9** (4)	7 - 34 **15** (15)	9 - 26 **15** (4)	2 – 30 **12** (5)	6 - 18 **11** (5)	8 - 15 **10** (3)	9 – 34 **15** (7)
Sr**	433 - 1512 **782** (279)	477 - 1582 **819** (819)	409 - 1319 **748** (259)	251 - 1855 **677** (239)	251 - 476 **409** (94)	420 - 830 **556** (141)	392 - 1130 **671** (222)
Li**	19 - 183 **31** (27)	22 - 67 **32** (32)	23 - 44 **30** (6)	21 – 189 **40** (22)	21 – 30 **26** (4)	21 – 60 **39** (14)	19 – 115 **41** (19)
Tl***	10 - 402 **161** (82)	100 - 727 **264** (264)	16 - 2340 **409** (439)	60 - 866 **338** (214)	209 - 540 **322** (149)	177- 691 **463** (191)	118 - 818 **337** (209)
U ***	4 – 231 **54** (61)	3 - 258 **36** (61)	1 - 185 **36** (44)	2 - 339 **71** (93)	7 - 369 **236** (198)	27 - 589 **167** (200)	1 – 324 **66** (105)
Pb**	1 - 13 **5** (2)	2 -12 **5** (5)	1 - 26 **4** (5)	1 - 88 **14** (19)	2 - 11 **7** (5)	2 - 18 **9** (6)	1 - 28 **11** (8)
As**	1 – 9 **3** (2)	1 – 6 **3** (3)	1 - 11 **3** (2)	1 - 11 **2** (2)	1 - 7 **5** (3)	2 - 18 **8** (6)	1 – 249 **15** (58)

*(mg L^{-1}). ** (µg L^{-1}). *** (ng L^{-1}).

On white wines, the concentrations of elements about red wines were lower. The decrease behavior in the concentration in geographical order from north to south was observed. From Rapel, were determined most of the elements in the lower concentration. Only Fe and U evidenced the higher content. In addition, the results from Itata reported the greater concentrations, except Zn and U. In Curicó, it was observed the minor concentrations of Mg, Fe, and Rb and the highest level of Ca, Zn and Tl. Samples from Maule were not evaluated.

In young wines according to the valleys Rapel. Curicó. Maule and Itata. The horizontal lines in each box plot represent from bottom to top the minimum value. The first quartile the median. The third quartile and the maximum value. Outlier values are indicated points outside the box. Values in the axis refer to log (E + 1). Log is the logarithm of the element concentration plus one.

Besides, the mean of grapes of the sample set was compared with for red and white wine. The evaluated red varieties were, Cabernet Sauvignon (n=33), Carmenere (n=21), Merlot (*n*=18), Syrah (*n*=11), Pinot noir (*n*=9), País (*n*=17), Cinsault (*n*=16) and Malbec (*n*=3).

The Anova was according to varieties and was analyzed, for Maule the K the Merlot 2318 mg L^{-1} was the highest value, while the lowest value was found in Itata in the wines Cabernet Sauvignon 798 mg L^{-1}. The elemental profile for Cabernet Sauvignon was between the intermediate values.

In Carmenere the major value is for Mg and Rb and minor value of Sr, Pb, Tl, and Li. While in the variety, merlot showed the lowest levels of Li. The Syrah wines were high in Tl and Sr, and they own the lowest in Rb and Ca. Pinot noir, meanwhile, has a long value of Zn and a small concentration of K and Mn. In País wines, it was determined K, Zn, and Mn as the highest concentration values and lowest value of Fe and Mg.

The variety Cinsault was established with a significantly greater difference in Na, Li, and Pb. In the case of Malbec wines, they present the highest value of As, Ca, Rb and Fe and the lowest value of Na and U. In the STable 3- 3 and STable 3- 4, there is a summary regarding red and white grapes.

Instead of the white wine grapes analysis, the set included 34 samples and three valleys. The mean of Sauvignon blanc (*n*= 6) and Chardonnay (*n*=5), from Rapel, Curicó and Itata valleys were studied and compared to the Muscat sparkling (*n*=5), Late harvest Muscat (*n*=3) and Muscat of Alexandría (*n*= 13) varieties, from Itata and also with viognier (*n*=2) from Rapel and Curicó. The levels for white wines are onto the permitted ranges for macro and micro minerals and trace element established by OIV [27]. The mean of all varieties of white wine was contrasted.

Viognier presented low levels of K 614 mg L^{-1}, Fe 593 mg L^{-1}; Sr 411 mg L^{-1}, Pb 3.5 µg L^{-1} and Tl 193 ng L^{-1} were found. EM contained the minor concentration in mean of Ca 37 mg L^{-1}, Na 8 mg L^{-1} and Zn 116 µg L^{-1} and the higher for Mg 344 mg L^{-1}, Rb 17 mg L^{-1} and Sr 858 µg L^{-1}. Muscat of Alexandria established the highest means in Na 33 mg L^{-1}, Fe 1490 µg L^{-1}, Li 45 µg L^{-1}, Pb µg L^{-1} and As 21 µg L^{-1}.

The samples of Late harvest Muscat include the long value in K 1105 mg L^{-1} and Mn 1502 µg L^{-1} and lower for As 0.7 µg L^{-1} and U 11 ng L^{-1} at Chardonnay superior means in Ca 108 mg L^{-1}, Zn 523 µg L^{-1} and Tl ng L^{-1}. On the contrary, the lowest concentration of Mg was 43 mg L^{-1}, Mn 910 µg L^{-1}, Rb 9 mg L^{-1} and Li 27 µg L^{-1}.

The Sauvignon blanc wines, the high levels for U 143 ng L^{-1} and low fo Mg 41 mg L^{-1}. The samples of Late harvest Muscat, Muscat sparkling and Muscat of Alexandria are from Itata and Chardonnay and Sauvignon blanc are the mean from the Rapel, Curicó, and Itata. Viognier wine is from Rapel and Curicó.

About the sources that contribute to the content of elements analyzed in our work in refer to the macronutrients K, Ca, Na, and Mg have a higher natural concentration [19].

However, they are often used in fertilizers and pesticides, as well as Zn, Mn, Fe and Pb [5]. The typical geogenic elements are As, Li, Sr, and Tl according to [23].

In industrial regions, the concentrations of Rb, Pb, As and Tl can be easily absorbed by plants from soil and aerial deposits. Further plants grew around coal power central and cement factories are most likely to have elevated levels of Tl [28] and several reports on the linear relationship between Arsenic contents of vegetation and concentrations in soils of both total and soluble species suggest that plants take up Arsenic passively with the water flow [28].

Additionally, the mean of grapes of the sample set for red and white wine was compared. The red wines varieties evaluated were Cabernet Sauvignon (*n*=33), Carmenere (*n*=21), Merlot (*n*=18), Syrah (*n*=11), Pinot noir (*n*=9), País (*n*=17), Cinsault (*n*=16) y Malbec (*n*=3). From the ANOVA analysis made for Cabernet Sauvignon, there were found higher values in comparison to other grapes in U with 70 mg L^{-1}. In Carmenere, higher values were found for Mg with 142 mg L^{-1} and Rb 15 mg L^{-1} and lower for Sr with 892 µg L^{-1}, Pb 4.2 µg L^{-1} and Tl 260 ng L^{-1}. Merlot contained the lowest concentration of Li 27.49 µg L^{-1}.

The samples of Syrah showed higher concentrations in Sr 892 µg L^{-1} and Tl with 547 ng L^{-1} and lower in Rb 9.5 µg L^{-1} and Ca 68 mg L^{-1}. In Pinot noir, the level of Zn was the highest with 463 µg L^{-1} and the lowest levels of K 1004 mg L^{-1} and Mn 1027 µg L^{-1}. For the País wine samples, the levels of K 1408 mg L^{-1} and Mn 2450 µg L^{-1} were the highest and Fe 1381 µg L^{-1} and Mg 104 mg L^{-1} were the lowest. In Cinsault, Na 26.56 mg L^{-1}, Li 52 µg L^{-1}, Pb 20 ng L^{-1} were superior, and As 1.61 µg L^{-1} was the lowest concentration. Malbec had the highest value for As 4 µg L^{-1} and the lowest of Na 8.48 mg L^{-1}.

For some elements, the content was higher or lower than the rest of the varieties in two grapes, Cabernet Sauvignon and Malbec with 110 and 131 mg L^{-1} for Ca. Carmenere and Malbec 15 and 14.5 mg L^{-1} for Rb, Merlot and Malbec for Fe 2228 µg L^{-1}, 2081 µg L^{-1} respectively, Syrah and Malbec for As with 2.93 and 4 µg L^{-1} were the highest concentrations. Zn, for País with 197 µg L^{-1} and Cinsault 166 µg L^{-1}, U in Syrah 25.93 ng L^{-1} and Malbec 22.45 ng L^{-1} the lowest concentrations.

When comparing the elemental profile of the grapes among the different valleys, we found that, in Maule, Merlot had the highest value of while the lowest value was for the Cinsault of the Itata 798 mg L^{-1}. On the other hand, the Ca with 212 mg L^{-1} in Cabernet Sauvignon of Curicó was the highest concentration and the lowest, 33.84 mg L^{-1} in the Syrah from Maule. The Mg in Carmenere 150 mg L^{-1} was the highest in Syrah and 33 mg L^{-1}. Merlot the lowest. The Cinsault from Itata showed the highest concentration of Na, 26.55 mg L^{-1} and the lowest was for Cinsault from Rapel with 7.20 mg L^{-1}.

The Merlot of Curicó showed the highest level of Fe with 2694 µg L^{-1} and the Syrah from Rapel with 1436 µg L^{-1} the lowest. For Mn, the País grape with 3907 µg L^{-1} from Maule corresponds to the highest value and 590 µg L^{-1} the lowest in Carmenere from Itata. The level of Zn in Pinot noir with 570 µg L^{-1} was the highest and 138 µg L^{-1} the lowest in the Carmenere from Itata. Sr, 1095 µg L^{-1} in Syrah from Curicó and 538 µg L^{-1} in Carmenere from Rapel, the highest and the lowest concentration respectively.

For Rb it was established 24.98 mg L^{-1}, the highest concentration value in samples of Carmenere from Curicó and 6.19 mg L^{-1} in Syrah of Rapel the lowest. The highest concentration of Li was found in Cinsault from Itata with 51.95 µg L^{-1} and the lowest in the Merlot of Rapel with 23.56 µg L^{-1}.

In the Syrah from Itata the level of Pb was 24.75 µg L^{-1} representing the highest value for this element and 2.11 µg L^{-1} in Merlot of Maule the lowest level of As of Syrah in Itata was the highest concentration with 28 µg L^{-1} and in the Pinot noir from Rapel the lowest value 1.59 µg L^{-1}.

From Itata the U showed the highest value with in Maule, 2.4 ng L^{-1} in Carmenere was the lowest concentration. The highest value of Tl was found in the Syrah grape of Maule with 1740 ng L^{-1} and the lowest in Carmenere of Rapel with 118 ng L^{-1}.

Instead of the white wine grapes analysis, the set included 34 samples of Muscat of Alexandria (*n*= 13), Sauvignon blanc (*n*= 6), Chardonnay (*n*=5), Muscat sparkling (*n*=5), Late harvest Muscat (*n*=3) and Viognier (*n*=2). The level for white wines is onto the permitted ranges stabilized by OIV.

For macro and micro minerals. As for trace element [42]. The comparison between the mean of varieties was contrast. For the Viognier presented, low levels of K 614 mg L^{-1}, Fe 593 mg L^{-1}, Sr 411 mg L^{-1}, Pb 3.5 µg L^{-1} and Tl 193 ng L^{-1} were found. The wines Muscat sparkling contained the minor concentration of Ca 37 mg L^{-1}, Na 8 mg L^{-1} and Zn 116 µg L^{-1} and the higher for Mg 344 mg L^{-1}, Rb 17 mg L^{-1} and Sr 858 µg L^{-1}. Muscat of Alexandría showed the highest results in Na 33 mg L^{-1}, Fe 1490 µg L^{-1} Li 45 µg L^{-1}, Pb µg L^{-1} and As 21 µg L^{-1}.

The samples of Late harvest Muscat included the long value in K 1105 mg L^{-1} and Mn 1502 µg L^{-1} and lower for As 0.7 µg L^{-1} and U 11 ng L^{-1}. In Chardonnay samples, the superior value in Ca 108 mg L^{-1}, Zn 523 µg L^{-1} and Tl ng L^{-1} were included. On the contrary, the lowest concentration of Mg 43 mg L^{-1}, Mn 910 µg L^{-1}, Rb 9 mg L^{-1} and Li 27 µg L^{-1} was appointed these samples. The Sauvignon blanc wines, the highest levels for U 143 ng L^{-1} and lowest for Mg 41 mg L^{-1} were reported.

The samples of Muscat of Alexandria, Late harvest Muscat and Muscat sparkling from Itata and Chardonnay and Sauvignon blanc are from Rapel, Curicó, and Itata. Viognier wines are from Rapel and Curicó.

3.3.2. Chemometric data analysis

Through the elemental concentration of wine samples, multivariate methods were developed for classification and prediction purposes. Then we used the PCA to further explore the data, a data preprocessing was needed to avoid the difference in the concentration of the original variables, and auto-scaling was used. PCA analysis showed that the first principal components explained less than 40%, the third component with 62% of the cumulative variance in the dataset.

Thereby, the model with the four valleys did not classify the samples in separate clusters in three dimensions. Our results are according to Šelih et al. [4], who have the similar geographical small distances between wine-growing valleys (distance between two points by Šelih within Slovenia was approximately 300 km).

In our valleys of study in Chile is within 86 km) hence, the differences in the content of the elements are relatively small when being compared to other countries, for example, Argentina, Spain, Italy, etc. In consequence, the discrimination between geographical origins of wines is more difficult, and although in our work data was explored among the local model groups, it was not possible to boost the percentage of variability of the first components, which shows a contribution of each one of the studied variables for the separation.

In all the combinations, the accumulated variance values were consistent to the general model. In contrast, in the study carried out by H. Hopfer et al. [1], whose distance between of vineyards from Northern California was 65 Km, they obtained result over 81% of the total variance ratio for red wines. This is possible perhaps because the multivariate analysis had a larger number of variables. 26 elements were described in dataset (B, Mg, P, Ca, Ti, Cr, Ni, As, Mo, Al, Co, Zn, Sr, Li, Be, Si, Mn, Rb, Cs, Er, Yb, Tl, Ga, Se, Ba, Eu). Thus, in future studies, it is necessary to include more variables to improve the classification of valleys and to find the characteristic elements for each valley.

A general model with four categories (valleys) was developed to supervise modeling. SIMCA and LDA were used. In the study, two sets of data were selected. 115 wines samples (Rapel *n*= 17, Curicó *n*= 17, Maule *n*= 21, Itata *n*=50) were used as modeling and 30 (Rapel *n*= 7, Curicó *n*= 5, Maule *n*= 6, Itata *n*=12) were used for external set.

Nevertheless, the result for the discrimination models is not sufficient for all valleys at the same time. According to SIMCA model, the cross-validation showed the percent of 56 for Rapel, 100 Curicó, 76 Maule and 96 for Itata, respectively for prediction. The highest value found for discriminant power by element was for Mn, Pb, Tl, Zn, Rb, and Li, then Li, As, Fe, K, U, Ca, Na, Sr, Mg and the power modeling value are distributed among all element. For LDA the coefficients with a higher value for classification function are represented in the function

$$276 + 14Na + 25K + 43Ca + 25Mg - 14Na - 0.6Zn + 55Mn - 13Rb - 37 + 70Sr + 30As$$

$$-6Tl + 18Pb + 2U$$

And the coefficient for discrimination

$$-0.3Na - 0.4K + 0.2Ca + 0.01Mg - 0.02Fe - 0.4Zn - 0.9Mn + 0.2Rb + 0.6Li + 0.019Sr - 0.4As$$
$$+0.4Tl + 0.3Pb + 0.1U$$

The patterns found for the discrimination for the two techniques are comparable. Then we infer that the most significant interactions among the valleys are about Mn, Li, Tl, Pb, Rb, and contributions distributed around the rest of the elements. Independently, for each valley for the classification according to the loading and scores analysis, the following characteristic patterns were found for Rapel, As, Li, Pb, U; Curicó Mn, Sr, Fe, Zn; Maule, Rb, K, Tl, Ca and Itata, Mg and Na.

The accumulated percentage of variance was similar to the first model made with all the samples described above. For this reason, the strategy, to enhance the percent was addressed to develop discrimination for six local models, Rapel and Curicó, Rapel and Maule, Rapel and Itata, Curicó and Maule, Curicó and Itata, Maule and Itata, divided in two sets, training set and external set. Towards the majority of these, the interclass distance was low, between 0.9 – 1.6, referring to the reduced separation between the valleys.

However, the result means for prediction was above 90% for cross-validation with SIMCA and LDA technique; and external validation 82% SIMCA, 75% LDA.

Values of cross-validation and external validation are shown in Table 3- 3. The acceptable percentage of correctly classified cases is presented for both techniques. On the other hands, according to SIMCA model decreasing order, the discriminant elements are for Rapel and Curico As, Tl, U, K, Mn, Na and Li, Rapel and Maule are, Tl, Li, Sr, As, Rb, Zn; Rapel and Itata Mn, Ca, Rb, Sr, As, Pb; Curico and Maule, Zn, K, Mg, Rb, Li, Tl, U; Curico and Itata, Mn, Pb, Zn, As, K; Maule and Itata, Mn, Fe, Rb, Sr, Mg, Pb, Li and Zn.

Table 3- 3. Results of the models found for the geographical origin classification of valleys: Rapel. Curicó. Maule and Itata with SIMCA and LDA with the percentage of cases correctly classified by cross-validation and external validation technique.

Valleys	**SIMCA**		**LDA**	
	cross validation	**External set**	**cross validation**	**External set**
Rapel Curicó	98%	83%	91%	84%
Maule Itata	97%	67%	90%	81%
Rapel Maule	93%	84%	94%	82%
Rapel Itata	95%	83%	93%	77%
Curicó Maule	100%	82%	94%	79%
Curicó Itata	100%	90%	91%	76%

3.3.3. Red and White wine samples models according to geographical origin and grape variety

About grape discrimination in red wines, PCA and SIMCA techniques were applied to analyze red wine grape. In total, 103 samples and five varieties were at the same time evaluated among the four valleys and in each valley the grapes Cabernet Sauvignon (*n*=31), Carmenere (*n*=21), Merlot (*n*=18); País (*n*=17) and Cinsault (*n*=16) were studied. From the exploratory analysis carried out with PCA, it was found an accumulated variance percentage in the first component similar to 68%, and in the third 84%.

The graphic representation of the scores shows the grouping made by the grapes from Curicó. Variables with more weight were identified for the separation. Among them, we found Sr, Fe, Zn, Li, Tl, Mn, Mg, U, Pb, and As. The rest of the valleys are grouped and superimposed according to the concentrations of Ca, K, Rb, and Na. From the discrimination model for this data set, low percentages were obtained.

Then, red wines models were evaluated. The accumulated variance of the PCA showed values no more than 60% for the group with all the valleys. It was not possible to establish groupings. However, percentages between 80% and 100% of prediction were calculated. In Rapel, Cabernet Sauvignon, Carmenere, and Merlot grapes were assessed.

The models of classification were less stable. The samples of Carmenere and Merlot could only discriminate the 71% and 67%, and the Cabernet Sauvignon 90%. The elements according to the SIMCA analysis with a higher contribution in the separation were Sr, Li, and K. In Curicó, Cabernet Sauvignon, Carmenere and Merlot grapes were studied, and the percentages of prediction were 100% for Cabernet Sauvignon and Carmenere and 66% for Merlot. Fe, K, Zn, and Rb were the elements with the highest discrimination value.

In Maule, the grapes Cabernet Sauvignon, Carmenere, Merlot, and País were considered. Among them, it was possible to distinguish the grouping of the País grape, mainly because of the concentration of Ca, Mn and Tl. The Cabernet Sauvignon, Carmenere and Merlot grapes, on the opposite, are found overlapped. The As and U concentrations contributed mainly to this grouping. For the classification, except for the Cabernet Sauvignon grape with 66%, all the samples were 100% correctly classified and for the prediction model, Ca, Mn and Tl were the principal elements.

For Itata valley, the PCA analysis of the samples of Cabernet Sauvignon, Carmenere, Merlot and País was not carried out. There were not observed patterns of separation among grapes, but they were 100% correctly classified for Cabernet Sauvignon, Carmenere and

Merlot. Cinsault and País only were able to predict the 56% and 67% of the samples. The elements with a higher contribution to the discrimination for Itata were K and Rb.

Cabernet Sauvignon, Carmenere and Merlot grapes were analyzed among the Rapel, Curicó, Maule, and Itata. The País wines samples were compared only among the Maule and Itata valley. The results for PCA and Cabernet Sauvignon showed an accumulated variance of 74% to 89% of the first and third factor. Nevertheless, well-defined groups were difficult to identify among the samples from Rapel, Maule, and Itata, which present overlapping.

The samples of Cabernet Sauvignon from Curicó successfully grouped, mainly for the concentration of Li, Tl, Mn, Sr, Zn, Fe and Mg. Rb y Li. A 100% of the correct classification was reached in the SIMCA model in the Rapel, Curicó and Itata valley and 85% in Maule. Rb and Li showed higher values of discrimination power for this model. With the Carmenere samples, it was reached a 79% in the first factor of PCA of explained variance and 90% in the third factor.

In this study, it could be differentiated the samples of Curico and Rapel. These groupings are due to the concentration of As, Na, and Pb for Rapel and Sr, Mn, Mg, Zn, Fe, Li, U and Tl for the Curico group. Maule and Itata grouped in only one, in function of the elements K, Ca and Rb. 100% of correct classification in the SIMCA model of the valleys Rapel, Curico, and Itata and 83% in Maule. Zn, Fe, and Sr showed power discrimination values for this model. From PCA to Merlot, the separation by valley was better defined.

The variance explained in the first factor was 81% and in the third factor 92%. The patterns of grouping were the following, for Rapel, Pb, As and Na; Curicó, Fe, Li, Sr, Zn, Mn, Tl, Mg and U. Maule, Ca; E Itata, K, and Rb. The model of classification SIMCA classified 100% of the samples for all valleys. The elements with higher power discrimination values were Li, Fe, Zn, and Pb. Between Maule and Itata, the País grape was compared. It was found a 96% of explained variance in the first PCA factor, the group of Maule was in function of the elements Ca, Na, Mn, Sr, Zn, Fe, Li and U, and Itata of Mg, Pb, Tl and As. The classification was satisfactory with a level of 100% of correct prediction.

The set of white wines in total are compound of 32 samples and six different grapes. The grapes used for the first model were: Sauvignon blanc (*n*=7), Chardonnay (*n*=5), Muscat of Alexandria (*n*=13), Muscat sparkling (*n*=5) and Late harvest Muscat (*n*=3). The result for PCA showed the variability around all the elements with the first factor 22% and third factor 53%. Two groups are formed. One, with the samples of Chardonnay and Sauvignon blanc, were grouped into the first and second main component (PC), related to the concentration of Zn, Ca y U.

The Late harvest Muscat and Muscat sparkling generated the second group in the first and third PC, associated with Mg, Sr, Li, Rb. For Muscat of Alexandria and Vigner to set, a cluster pattern is not possible.

In the classification analysis with SIMCA, the result of cross-validation for Sauvignon blanc samples was 86% and for the other varieties was 100% of correct prediction. The principal elements selected for the model were U, Mg, Na, and Rb.

3.4. Conclusions

The determination of the inorganic composition of wine samples from four important wine making valleys of central and southern Chile combined with chemometric treatment provides classification models to achieve an acceptable differentiation of geographical origin of the so-called "young and monovarietal wines." The combination of PCA and SIMCA treatment of data set, the patterns for valleys are not possible to propose.

Although in our study it is mentioned the separation patterns, the contribution of each element is significant and therefore, the increase in the number of variables investigated to increase the classification models is required.

The prediction results indicate acceptable outcomes. These models can discriminate the valleys reasonably by geographical areas. The composition of the major elements investigated for wines in the four Chilean winemaking regions shows enough similarity between the concentrations of these elements as well as significant differences to recognize the four valleys.

Thus, it can be concluded that the models generated by LDA and SIMCA are efficient to predict and acceptably classify the wines according to their geographical origin considering that along the territory there are no climatic or geographical differences compared to the recognized differences between East and West.

Similarly, for the classification models of the varieties of red and white grapes. In the study, it was addressed to separate Itata and Curicó valleys, but the interclass distance was low for the other valleys under study.

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3.6. Conflicts of interest

The authors declare no competing financial interests.

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Supplementary Material

STable 3- 1. Instrumental and optimized operating conditions

	Elements				
Parameters FAAS	**Fe**	**Mn**	**Zn**	**Mg**	**Ca**
Filament current (mA)	30	20	10	6	12
Wavelength (nm)	248	279	214	258	422
Slit (nm)	0.2	0.2	0.7	0.7	0.7

	Elements				
Parameters FAES	**Sr**	**Na**	**Li**	**K**	**Rb**
Filament current (mA)	20	15	15	20	20
Wavelength (nm)	460	589	670	780	766
Slit (nm)	0.2	0.2	0.7	0.7	0.7

STable 3- 2. Concentration range of calibration curves

	Range [mg L^{-1}]	**Calibration curve**	**R^2**	**LOD [mg L^{-1}]**	**LOQ [mg L^{-1}]**	**RSD**
Li	1.0 - 2.5	y = 0.649x - 0.010	0.999	0.002	0.007	0.009
Na	0.1 - 0.8	y = 0.212x + 9.44x10-5	0.999	0.004	0.012	4.520
K	1.0 - 2.0	y = 0.609x - 0.019	0.996	0.007	0.025	12.040
Rb	2.0 - 6.0	y = 0.179x - 0.116	0.998	0.024	0.080	0.000
Zn	0.4 - 0.8	y = 0.303x + 0.002	0.999	0.004	0.015	55.900
Fe	1-0 - 4.0	y = 0.052x + 0.003	0.999	0.026	0.08	223.610
Mn	0.5 - 2.0	y = 0.104x+ 0.002	0.999	0.013	0.043	100.000
Ca	2.0 - 5.0	y = 0.059x - 0.005	0.999	0.043	0.142	22.020
Mg	0.1 - 0.5	y = 0.827x - 0.002	0.999	0.002	0.007	34.230
Sr	1.0 - 4.0	y = 0.345x- 0.003	1.000	0.006	0.021	2.790

STable 3- 3. Concentration range. Means (in bold) and standard deviations (values in parenthesis) of minor and trace elements found in red varieties.

	Red wine (*n*=22)							
	Cabernet Sauvignon (n=10)	***Carmenere (n=21)***	***Merlot (n=18)***	***Syrah (n=11)***	***Pinot noir (n=9)***	***Malbec (n=4)***	***Pais (n=17)***	***Cinsault (n=16)***
K *	646 -2482	667 - 3071	595 - 2840	707 - 1898	484 - 1505	689 - 2079	454 - 3321	823 – 1948
	1202 (435)	**1333** (546)	**1281** (582)	**1211** (375)	**1004** (351)	**1341** (653)	**1408** (814)	**1193** (308)
Na *	1 - 62	2 - 55	5 - 53	10 - 32	2 - 49	2 - 17	7 - 42	6 – 93
	13 (13)	**19** (13)	**14** (11)	**18** (6)	**19** (14)	**8** (6)	**19** (10)	**27** (27)
Ca *	30 - 293	16 - 182	38 - 188	24 - 191	30 - 215	83 - 188	25 - 190	23 – 284
	110 (74)	**75** (50)	**83** (49)	**68** (59)	**103** (53)	**132** (56)	**76** (49)	**71** (60)
Mg *	20 - 437	28 - 292	24 - 287	29 - 270	22 - 284	22 - 260	15 - 378	27 – 286
	134 (101)	**142** (82)	**137** (94)	**141** (84)	**132** (80)	**112** (111)	**105** (94)	**116** (71)
Zn **	58 - 930	21 - 565	173 - 519	197 - 666	286 - 760	152 - 774	18 - 642	19 – 389
	372 (188)	**316** (166)	**340** (95)	**392** (164)	**463** (168)	**390** (268)	**197** (146)	**166** (115)
Mn **	367 - 4678	470 - 3300	701 - 3899	710 - 2781	486 - 1781	1050 - 1531	207 - 11762	358 – 6630
	1513 (933)	**1217** (659)	**1625** (731)	**1669** (632)	**1027** (428)	**1385** (226)	**2450** (2791)	**1809** (1781)
Fe **	212 -3460	449 - 3994	1340 - 3814	788 - 2538	558 - 3519	853 - 3167	692 - 3460	635 – 3090
	1800 (930)	**1704** (985)	**2228** (710)	**1707** (590)	**1912** (110)	**2082** (984)	**1381** (798)	17**11** (729)
Rb *	2 - 20	3 - 34	6 - 22	3 - 17	2 - 17	12 - 17	5 - 26	6 – 20
	13 (4)	**14** (8)	**13** (4)	**9** (4)	**11** (5)	**15** (2)	**12** (5)	**12** (3)
Sr **	409 - 1274	453 - 1171	518 - 1357	470 - 1581	374 - 1232	652 - 976	251 - 1855	473 – 1260
	708 (210)	**624** (202)	**822** (251)	**892** (392)	**698** (231)	**833** (135)	**830** (388)	**705** (186)
Li **	21 - 45	21 - 183	19 - 61	21 - 51	22 - 39	24 - 39	29 - 71	28 – 189
	30 (7)	**37** (35)	**27** (9)	**30** (8)	**28** (6)	**31** (6)	**40** (12)	**52** (38)
Tl ***	60 - 755	10 - 866	55 - 942	92 - 2340	73 - 788	198 - 497	16 - 765	125 – 448
	277 (175)	**263** (217)	**278** (241)	547 (660)	330 (242)	301 (135)	300 (226)	311 (113)
U ***	3 - 338	1 - 225	1.4 - 240	2 - 104	4 - 310	3 - 40	3 - 330	2 – 167
	70 (86)	**44** (57)	**45** (60)	**26** (31)	**70** (96)	**22** (18)	**67** (92)	**57** (53)
Pb **	1 - 12	1 - 15	0.7 - 13	3 - 36	1 - 12	0.5 - 7	0.4 - 42	1 – 83
	5 (2)	**4** (3)	**4** (3)	**7** (10)	**6** (3)	**3** (3)	**10** (11)	**20** (25)
As**	0.6 - 8	0.4 - 7	0.6 - 9	1 - 11	0.5 - 6	0.9 - 7	0.6 - 11	0.2 – 5
	3 (2)	**3** (2)	**3** (2)	**3** (3)	**2** (2)	**4** (3)	**2** (3)	**2** (1)

*(mg L^{-1}). ** (µg L^{-1}). *** (ng L^{-1}).

STable 3- 4. Concentration range. Means (in bold) and standard deviations (values in parenthesis) of minor and trace elements found in white varieties.

	White wine		
	Chardonnay (n=5)	***Viogner (n=2)***	***Sauvignon blanc (n=6)***
K*	797 - 1176	455 - 773	535 – 1069
	1017 (162)	**614** (225)	**713** (192)
Na*	11 - 19	17 - 21	7 – 23
	15 (3)	**19** (3)	**13** (6)
Ca*	16 - 264	57 - 93	43 – 176
	109 (97)	**75** (25)	**96** (46)
Mg*	7 - 89	88 - 139	22 – 70
	43 (37)	**114** (36)	**41** (17)
Zn**	420 - 713	199 - 533	293 - 556
	523 (113)	**366** (236)	**391** (104)
Mn**	329 - 1310	903 - 1114	207 - 1354
	910 (402)	**1009** (149)	**797** (403)
Fe**	641 - 2173	308 - 878	314 - 1455
	1447 (710)	**593** (403)	**1030** (412)
Rb*	6 - 15	8 - 11	10 – 18
	9 (3)	**10** (2)	**14** (3)
Sr**	322 - 484	251 - 572	330 - 564
	434 (68)	**412** (227)	**444** (76)
Li**	21 - 38	24 - 34	19 – 60
	27 (7)	**29** (7)	**35** (18)
Tl***	290 - 691	177 - 209	141 - 818
	541 (155)	**193** (23)	**396** (241)
U ***	38 - 340	7 - 233	10 - 360
	158 (122)	**120** (160)	**144** (139)
Pb**	2 - 14	2 - 5	0 .3 - 18
	7 (5)	**4** (2)	**9** (7)
As**	1 - 6	3.5 - 4	0.1 – 18
	4 (2)	**4** (0)	**6** (6)

*(mg L^{-1}). ** (µg L^{-1}). *** (ng L^{-1}).

STable 3- 5. Comparison between the found p values for F-test by concentration for Ca, Fe and K in certified reference material, with actual concentration.

Element	CRM* [mg/L]	Real [mg/L]	*p***
Ca	74.4 ± 3.0	46.4 ± 1.0	0
Fe	2.57 ± 0.1	2.61 ± 0.1	0.37
K	987 ± 30	970 ± 31	0.18

* certified reference material

** $p < 0.05$ indicates statistically significant at a 95% confidence difference.

4. Elemental charge and assessment of metal-organic compounds complexes in Chilean natural wines by two-dimensional chromatography and ICP-MS.

Title: Elemental charge and assessment of metal-organic compounds complexes in Chilean natural wines by two-dimensional chromatography and ICP-MS.

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Abstract

Itata Valley emergent wine making areas in Chile and new trends in winery practices, as well as growing grapes with minimum technological and chemical intervention, are addressed for the marketing new value-added products such as “natural wines.”

In this communication, inductively couple plasma – mass spectrometry (ICP-MS) has been used for assessing total contents of trace and ultra trace elements in wines, but also for assessing metals-low molecular weight organic compound (LMWC) complexes after two-dimensional chromatography [size exclusion chromatography (SEC), and ionic exchange chromatographic (AEC)] hyphenated with ICP-MS.

A Superdex Peptide 10/300GL (SEC) was first used for separating low molecular weight compounds in natural wines (nine fractions of molecular weights ranging from 200 to 6690 Da monitored by UV detection). Elements such as B, Cu, Ni, Rb, and Ti were found to be bounded to compounds of molecular weights within the 200-2000 Da range. This SEC fraction was further characterized by AEC (use of PR-X100 column), and three compounds (UV detection), F1, F2 and F3 (retention times of 2.6, 3.2, and 3.8 min) were observed.

AEC-ICP-MS showed the presence of B-, Rb-, and Li-LMWC complexes. B was found to be bounded to LMWC in fraction F2; whereas, Li was associated to fraction F1. However, other elements such as Rb and Mn, which were associated with the investigated SEC fraction, were not observed after AEC-ICP-MS analysis. These finding could imply high lability of Rb- and Mn-LMWC complexes.

Several white and red wines were analyzed for total metal contents and also for metals associated with 200-2000 Da SEC fraction. Average concentrations of 0.053 $\mu g\ L^{-1}$ (Li), and 30.3 $\mu g\ L^{-1}$ (B) were found. *Keywords:* Wine, metal, chemometrics, complexes.

4.1. **Introducción**

Itata Valley is one winemaking emerging area in Chile and new trends in winery practices as well as growing grapes (minimum technological and chemical intervention) are addressed for the marketing new value-added products. This area is located between 36 ° 32'00'S; 72 ° 26'00'W and 36 ° 44'00 " S; 72 ° 28'00"W [1], in which three geographic macro forms establish a denomination around the wines generated in these zones, which vary according to latitude [2]. To the west is the mountain range of the coast, which has surroundings to the Pacific Ocean, followed by the intermediate valley and to the east the Andes mountain range.

Several authors have investigated the capacity of metals as an important indicator of geographical differences in the past decade [3–10]. Also, metals are important in wine because they participate in several reactions that determine the final quality regarding organoleptic, nutritional properties and stability [11,12]. On the other hand, due to reductive characteristics of wine [13], metals can be found, in the form of free ions, in complexes with organic acids or complexes with peptides and/or macro molecules including pectic polysaccharides, proteins and polyphenols [14–16].

Several chemical forms for elements, mainly Cu, Fe, Mg, Zn, Mn, Ca, Sn, Se, Cr, As, among others, have been established by several methodologies, generally hyphenated strategies, because they offer separation capacity of the species in combination with high-sensitivity detection by atomic mass spectrometry [16,17]. Other techniques include fractionation through ultrafiltration, which allows the determination of the distribution of different particle size fractions [18,19]. Also, commercial resins – Amberlite® XAD-8, Dowex 50-x8 and Dowex 1-x8, Amberlite® XAD2 adsorbent minicolumn [18–27], through a C18 and cation exchange SPE cartridge [16], and Donnan Membrane Technique (DMT) [28] have also been reported.

In the present study, to generate chemometric models of classification and prediction according to the denominations by macro geographical forms of the wine growing areas from Itata valley, several metals were quantified. Additionally, metal complexes of organic compounds of low molecular weight were examined using two-dimensional chromatography and ICP-MS analysis.

4.2. Materials and methods

4.2.1. Instrumentation

Metal content was measured with a Perkin Elmer Nex-Ion 300X ICP-MS (Waltham, MA, USA) equipped with a SeaFast SC2 DX autosampler (Elemental Scientific, Omaha, NB, USA). The argon gas with a minimum purity of 99.996% was supplied by Air Liquide. An HF- resistant and high-performance perfluoracetate (PFA) nebulizer PFA-ST model, coupled to a quartz-made baffled cyclonic spray chamber, cooled with The PC3 system from ESI was used. The chromatographic system was an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA) equipped with Superdex peptide 10/300GL SEC column (30 cm × 10 mm I.D., optimum separation range for peptides between 100 and 7000 Da) from GE Healthcare BioSciences AB (Uppsala, Sweden) and a PRP-X100 (250 mm × 4.1

mm I.D.) from TosoHaas (Tokyo, Japan). UV-visible index detector was used. 250µL Hamilton Gastight 1725 syringe (Bonaduz, Switzerland) was used for manual injection. An ORION 720A plus pH–meter with a glass–calomel electrode (ORION, Cambridge, UK), 0.45 µm polycarbonate membrane Nucleopore filters (Millipore), and Albet®LabScience 0.20 µm cellulose acetate syringe filters (25 mm diameter) from Albet-Hahnemuehle (Dassel, Germany).

4.2.2. Reagents

Deionized water 18.2 MΩ cm, produced by MilliQ Gradient A1, USA. Standard solutions used for the calibration curves set were prepared by dilution of stock analytical standards (1000 mg L^{-1}) supplied by Merck (Poole, Dorset, UK). Multi-element standard solution 1; single-element Hg, Ge, Te, Y, Sc, Rh provided by Perkin Elmer Pure Plus. Nitric acid Suprapur (69% v/v) and high purity ethanol from (Pamreac). Ready ReadyCalkit PEO/PEG containing polyethylene oxides (molecular weights of 6.55, 17.9, 40.1, 87.8, and 220 kDa) were from PSS Polymer Standard Services GmbH (Mainz, Germany). Blue dextran 2000 (molecular weight 2000 kDa) was from Pharmacia Biotech (Piscataway, NJ, USA). Other reagents were sodium azide (Merck), sodium hydroxide (Merck). Ammonium sulphate (Panreac) and diammonium hydrogen phosphate (BDH, Poole, UK). Sodium hydrogen carbonate was from Merck. Nitric acid 69% was from Panreac (Barcelona, Spain).

4.2.3. Wines samples

Eighty-three varietal and young wine and organic wine samples from were obtained from among the parallel 36° and 37° in the denominated Itata valley from south region of Chile (traditional small vineyard located between the Andes Mountains, central valley and the Coast Mountain, distance within east to west approximately 65 Km). The wines were directly sampled from the stainless steel fermentation tanks, before the final process of winemaking (*e. g* without malolactic products, bentonite products, gelatin products, etc) and after of alcohol fermentation. Table 4- 1 summarizes a brief description of wine samples. The sample set from five vineyards were elaborated from six red grape: Cinsault, Pinot noir, Cabernet Sauvignon, País, Carmenere and Malbec. Also, three white varieties, Muscat of Alexandria, Chardonnay, and Sauvignon blanc were also considered in the study. Wine samples were collected in polypropylene bottles and stored at 5 °C. Each sample was analyzed in duplicate and analyzed in triplicate.

Table 4- 1. Wine simple identification.

Denomination	**Varietal**	**Type**
Coast		
	Cinsault	Red
	Cabernet Sauvignon	Red
	Carmenere	Red
	Pinot noir	Red
	Muscat of Alexandria	White
Central depression (valley)		
	Cinsault	Red
	Cabernet Sauvignon	Red
	País	Red
	Chardonnay	White
	Muscat of Alexandria	White
Andes Mountain		
	Cinsault	Red
	Cabernet Sauvignon	Red
	Carmenere	Red
	Malbec	Red
	Sauvignon blanc	White

4.3. Analytical procedures

4.3.1. ICP-MS measurements

Metals in wine were measured under standard operating conditions described in STable 4- 1. The measured isotopes, in order of mass number, were: ^{7}Li, ^{9}Be, ^{11}B, ^{23}Na, ^{24}Mg, ^{26}Mg, ^{27}Al, ^{31}P, ^{39}K, ^{43}Ca, ^{47}Ti, ^{51}V, Cr, ^{54}Fe, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{65}Cu, ^{66}Zn, ^{75}As, ^{77}Se, ^{78}Se, ^{88}Sr, ^{98}Mo, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{138}Ba, ^{195}Pt, ^{202}Hg, ^{205}Tl and ^{208}Pb. All samples were filtered through 0.20 µm cellulose acetate syringe filters (Millipore) before ICP-MS measurements. Determination of wine samples was studied by standard addition method and sample treatment by 1:10 dilution with HNO_3 [1% v/v]. For potassium and calcium determination, a 1:100 dilution was necessary.

Volumetric equipment was soaked in 1% distilled HNO_3 overnight and thoroughly rinsed with Milli-Q water, before use. High purity 18.2 MΩcm water was used in the preparation of all solutions. High-purity ethanol was used for preparing matrix-matched standards. The calibration range for K, Ca, Mg, Al, Fe, Na, and Sr were within the 0 to 10 mg L^{-1} range; whereas, for trace elements Li, Be, B, V, Cr, Ti, Se, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sn, Sb, Ba, Pt, Tl and Pb, calibration was up to 200 µg L^{-1}. For Hg determination, calibration was performed within the 0 - 10 µg L^{-1} range. Blank solutions consisted of solutions containing the same ethanol volumes that those found in wines. Tellurium, yttrium, indium, germanium, rhodium, and scandium were selected as internal standards at 10 µg L^{-1}.

Accuracy was assessed by the analytical recovery assay. Therefore, samples were spiked with the analytes to final concentrations of 1, 10 and 100 µg L^{-1}. In all cases, significant recoveries between 80 and 140% were obtained.

The LODs (3 SD criterion, S.D. standard deviation of eleven measurements of a reagent blank) and LOQs (10 SD criterion) was used. The elements analyzed for red and white wines were Li, Be, B, Na, Mg, Mg, Al, P, K, Ca, Ti, V, Cr, Fe, Mn, Fe, Co, Ni, Cu, Zn, Cu, Zn, As, Se, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Pt, Hg, Tl and Pb. Summary of LOD and LOQ are shown in Table 4- 2. Metals bound to low molecular weight organic compound (LMWC) were assessed by using an aqueous calibration matched with the mobile phase (25 mM/25 mM ammonium sulphate/ammonium hydrogen phosphate, pH 6.5), with trace metal concentrations from 0 to 200 μg L^{-1}. The SEC fractions (2 mL) were diluted to 6 mL with HNO_3 [1% v/v], and the same internal standards were used for calibration. Table 4- 3 shows the average concentration and standard deviation of the quantified elements in wines.

4.3.2. SEC-HPLC-UV/ICP-MS measurement procedure

Two different analytical strategies for assessing metal-LMWC were used. The first one was SEC-HPLC-UV / ICP-MS techniques. Separation was performed under isocratic elution conditions (flow rate set at 0.9 mL min^{-1}) using a mobile phase of 25 mM/25 mM ammonium sulphate/ammonium hydrogen phosphate buffer solution at pH 6.5. SEC column calibration was performed with PEG/PEO Kits (molecular weights range 232 – 1015000 Da). The exclusion volume (V_0) was determined using Blue dextran, while the permeation volume (Vp) was fixed by injecting Milli-Q water. Measurements were performed using an injection volume of 20 µL and detection UV 205 nm.

4.3.3. AEC-HPLC-UV/ICP-MS measurement procedure

LMWC fractions collected from SEC were then subjected to AEC- HPLC-UV and ICP-MS detection. Separation was performed under isocratic conditions (25 mM / 25 mM ammonium sulphate/diammonium hydrogen phosphate buffer solution, pH 6.5, as a mobile phase, flow rate of 1 mL min^{-1}). The UV detection was monitored at 205 nm. When coupling the AEC-HPLC with ICP-MS for metal detection, the operating ICP-MS conditions listed in STable 4 - 2 (adapted from the protocol by [29]).

Table 4- 2. Trace elements LOQ and LOD determination in red and white wines.

Red [µg L^{-1}]					**White [µg L^{-1}]**				
Elements	***(a.m.u)***	***LOQ***	***LOD***	***%RSD***	***Elements***	***(a.m.u)***	***LOQ***	***LOD***	***%RSD***
Li	7	2.4	0.02	76	**Li**	7	0.2	0.05	1.6
Be	9	0.12	0.04	153	**Be**	9	0.05	0.01	0.5
B	11	10	3.4	61	**B**	11	8	3	84
Na[a]	23	3	0.6	185	**Na**[a]	23	2	0.5	17
Mg[a]	24	1.4	0.4	84	**Mg**[a]	24	0.1	0.03	1
Mg[a]	26	1.7	0.5	87	**Mg**[a]	26	0.1	0.03	1
Al[a]	27	0.3	0.08	122	**Al**[a]	27	0.1	0.04	1.3
P[a]	31	8	2	1	**P**[a]	31	1.4	0.4	14
K[a]	39	28	9	233	**K**[a]	39	2.0	0.6	20
Ca[a]		30	9	5	**Ca**[a]		32	10	3
Ti	47	2.7	4.8	1.5	**Ti**	47	4.9	1.5	36
V	51	8	0.07	143	**V**	51	2	1	20
Cr	52	13	0.6	66	**Cr**	52	2	0.5	15
Fe	54	183	49	130	**Fe**	54	57	17	57
Mn	55	5.7	0.1	22	**Mn**	55	1.5	0.4	13
Fe	57	56.1	16.8	22	**Fe**	57	53	16	53
Co	59	1.2	0.3	120	**Co**	59	0.1	0.02	0.6
Ni	60	1.9	0.08	148	**Ni**	60	3	1	24
Cu	63	8.5	1.5	223	**Cu**	63	6.8	2.0	42
Zn[a]	64	0.5	0.2	96	**Zn**[a]	64	0.2	0.1	2
Cu	65	8.3	0.1	184	**Cu**	65	6.7	2.0	42
Zn[a]	66	0.6	0.26	114	**Zn**[a]	66	0.3	0.1	2
As	75	1.9	0.6	217	**As**	75	0.3	0.1	3
Se	77	4.3	1.3	160	**Se**	77	7	2	55
Se	78	4.3	1.3	130	**Se**	78	3	1	25
Sr	88	8	2	146	**Sr**	88	0.03	0.01	0.3
Mo	98	3	1	56	**Mo**	98	2	1	20
Ag	107	1.0	0.3	93	**Ag**	107	1.0	0.3	11
Cd	111	0.7	0.2	115	**Cd**	111	0.2	0.1	2
Sn	118	12	0.2	213	**Sn**	118	7	2	72
Sb	121	4	0.08	161	**Sb**	121	2	1	25
Ba	138	1	0.2	145	**Ba**	138	1	0.3	11
Pt	195	0.07	0.02	142	**Pt**	195	0.07	0.02	1
Hg	202	8	2	225	**Hg**	202	4	1	30
Tl	205	0.04	0.01	169	**Tl**	205	0.04	0.01	0.5
Pb	208	1.3	0.4	182	**Pb**	208	1.3	0.4	13

[a] Concentration (mg L^{-1})

Table 4- 3. Summary of wine samples composition obtained with ICP – MS/MS

***All wine samples* µg L^{-1}**			
Elements	**Range**	**Mean**	**RSD**
^{7}Li	1.0 - 15	4.9	3.3
^{9}Be	0.1 - 0.8	0.2	0.1
^{11}B	247 - 16471	6065	3254
^{23}Na[a]	3.1 - 63	12	13
^{24}Mg[a]	62 - 335	138	64
^{26}Mg[a]	66 - 294	135	55
^{27}Al	105 - 1414	418	240
^{31}P[a]	45 - 409	137	104
^{39}K[a]	331 - 1071	694	174
^{43}Ca[a]	51 - 105	72	12
^{47}Ti	121 - 990	420	260
^{51}V	0.7 - 5	2.8	5.1
^{52}Cr	390 - 817	587	86
^{54}Fe	263 - 5543	1843	1210
^{55}Mn	283 - 3017	1651	674
^{57}Fe	263 - 5514	1847	1210
^{59}Co	0.1 - 14	2.7	2.4
^{60}Ni	5 - 45	16	9.6
^{63}Cu	16 - 300	71	52.9
^{64}Zn	50 - 836	259	209
^{65}Cu	19 - 238	67	49
^{66}Zn	50 - 2495	415	528
^{75}As	0.3 - 7	0.7	0.8
^{77}Se	1.0 - 10	4.4	1.9
^{78}Se	2.8 - 6.5	4.6	1.2
^{88}Sr	109 - 1410	633	445
^{98}Mo	0.3 - 8	1	3
^{107}Ag	0.7 - 5	1.7	0.9
^{111}Cd	0.1 - 1.3	0.2	0.3
^{118}Sn	0.3 – 8.9	2	1.5
^{121}Sb	0.1 – 4.9	0.5	0.8
^{138}Ba	32 - 730	368	236
^{195}Pt	0.01 - 1	0.1	0.2
^{202}Hg	0.3 - 3.5	0.7	0.5
^{205}Tl	0.1 - 1.1	0.4	0.3
^{208}Pb	1.0 - 58	9	14

[a] Concentration (mg L^{-1})

4.3.4. Data analysis

The chemometric techniques used in this study were an analysis of variance (ANOVA), PCA, SIMCA, and LDA. For multivariate data analysis, the statistical packages Infometrix Inc, with the software Pirouette ver. 4.0, Statgraphics Centurion, version 16.1 (2010) and XLSTAT version 1 (2016) were used.

4.4. Results and discussion

A comparative evaluation of calibration methods (1:10 dilution in 1% (v/v) HNO3, a variable amount of ethanol corresponding volume of alcohol dilution of samples, and the standard addition method) was performed. In all cases, high sensitivity was obtained when using the standard addition method. These results agree with those reported by [10,30]. The data are inside the ranges established by several authors and by the International Organisation of Vine and Wine (OIV), STable 4- 3. The eighty-three samples of monovarietal wines were analyzed in triplicate by using the standard addition methods. Good linearity (R2 ≥ 0.999) and repeatability (relative standard deviation [RSD] ≤ 12%) were achieved.

4.4.1. An inorganic fraction in wines

Elements such as V, As, Cd, Sn, and Hg were found to be below the limit of detection using this method in all analyzed samples. Elements such as Na, Mg, K and Ca were present at high concentrations (higher than 10 mg L^{-1}). Fe, Mn, Al, Cu, Zn, B, Sr, and Rb were found in trace levels, above 0.1 mg L^{-1}, and ultra-trace concentrations of the remaining elements were found Li, Sc, Ti, V, Cr, Co, Ni, As, Se, Mo, Ag, Cd, Sn, Sb, Ba, rare earth, Tl, Pb, etc., lower than 0,1 mg L^{-1}. Some metals are in higher proportion to red wines, the fact that could be mainly influenced by the vinification process [31].

Data set were evaluated by categories, the ANOVA, and the *F* - the reason of the results was calculated (STable 4- 4, STable 4- 5), and the most important variables were selected. Element below the LOD was not considered in the study. The geographic division between coast, valley, and Andes is determined mainly by Zn (7.4), B (6.4), Tl and Zn with (5.9), P (4.1), Ba (3.6), Sb (3.1), Co (2.6), Al (2.5), Cr (2.3), Mg, Mo and Pb with (2.0), Mg (1.9), Ca (1.5), Mn (1.3) and Ni (1.1). These elements showed the major *F*- reason values (in parentheses), with a statistically significant difference with 95% of confidence.

Furthermore, the higher concentrations of Zn, B, Tl, Mn, Ni, and Cr were found in the Andes zone; whereas, the lowest values of Zn, Tl, P, Co, Al, Cr, Mg, Mn and Ca and the highest concentrations of Ba, Sb, Mo, and Pb were found in Coast zone. Wines from the intermediate depression, moreover, showed the highest levels of P, Co, Al, Mg and Ca, and the lowest levels of B, Sb, Ni, and Pb. The concentration levels according to these categories are shown in Table 4- 4.

Almost the vineyard with red wines the classification involved five vineyards across the Itata valley named Neira (N), Piedras del Encanto (Pe), Puertas del Itata (PI), Entre valles (EV) and Chillan (Cll) Table 4- 5. Twenty-seven element have the significant difference, and the elements Na (59), ^{64}Zn (19), Sr (9), Ba (8), Cr (7) and B (5) were the most important according to with ANOVA. Among the most noteworthy data, the lowest values of Mg, Al, Fe, Mn, Pt, Zn, Tl and Pb were found in the EV vineyard. The differentiation of Andes zone was made by Cll vineyard; the highest levels of the elements were found in this area.

The concentration of Pb in Pu and Cll showed the highest values. While for the vineyards that provided white wines, see Table 4- 6, (PI, Pe and Cll), the elements Pb (5062), Ba (3842), Cu (2621), Mn (1643), Li (769), B (460), ^{54}Fe (293), ^{57}Fe (292), ^{31}P (258), Sr(229), Ni (157), ^{64}Zn (142) and ^{66}Zn (126) had the highest values in Anova.

Between the groups of white wine grapes. In table STable 4- 6 showed the concentrations of higher white grapes (Chardonnay [Ch], Sauvignon blanc [SB] and Muscat of Alexandria [M]) and which higher levels were observed. For the variety Ch the K, Ca, V, Fe, Co, Ni, Zn, Se, Cd, Sb, and Pb. In variety SB the elements B, Na, Mg, Al, P, Ti, Cr, Mn, As, Sr and Tl were larger; as for grape M were Li, Se, Mo, Ag, Sn, Ba, and Hg.

The elemental relationship between red and white varieties was inverse in agreement of *F*- reason values, and, only Pt, Mo, Ca, Se and Be had *p*- values above 0.05.

Six red grape varieties (see STables 4- 7 and 8) (Cinsault [Csl], Pinot noir [Pn], Cabernet Sauvignon [CS], País [P], Malbec [Mlb], Carmenere [Cmn], were studied. For these two groups, we found more variables with significant differences. For Mlb, the elements in higher concentrations were (Mg, Al, P, Ca, Cr and Zn); in Cmn (B, Ni, Cu, Zn, Tl, and Pb). Moreover, the PB grape was characterized by containing the lowest levels of P, K, Ca, Cr, Zn, Se, and Tl. Cls the highest levels of Se, K, Li and Sb and the lowest concentrations of Mg, Al, Fe, and Ni were determined. For CS the highest concentrations of Be, Ti and Fe were observed.

From the results with white wine grapes, the difference in concentrations increase significantly the difference between them and red grape wines. Among the group of white grapes for Ch shows mainly higher levels of Fe, Co, Ni, Zn, Cd, and Pb; whereas, M shows high values of Cu, Sn, Sb, Ba and Hg. In SB high values of B and Mn, the higher levels were determined. The lower concentrations considered for Ch were Mn and Ba. As regards to M the lowest concentrations of B, Fe, and Ni, were found. According to the relation calculated for the different selected categories, the principal differentiation observed was related to white wines.

Table 4- 4. Summary of wine samples composition obtained with ICP – MS/MS by geographic denomination.

Element	**Coast**	**valley**	**Andes**
	(*n* = 38)	(*n* = 16)	(*n* = 24)
^{7}Li	1.5 – 11 **5** (2)	1.3 - 5 **3.5** (1.8)	1.3 - 15 **5** (5)
^{9}Be	0.10 - 0.6 **0.2** (0.1)	0.1 - 0.8 0.2 (0.2)	0.10 - 0.5 0.2 (0.2)
^{11}B	2995 – 8604 **5816**(2084)	998 – 7370 **3850** (2326)	247 – 16471 **7436** (3986)
^{23}Na^{a}	3 – 63 **14** (17)	4 - 10 **7** (2)	4 – 24 **12** (6)
^{24}Mg^{a}	62 – 152 **115** (33)	88 - 335 **181** (108)	77 – 280 **138** (40)
^{26}Mg^{a}	66 – 150 **117** (30)	84 – 294 **181** (92)	88 – 268 **133** (35)
^{31}P^{a}	45 – 270 **87** (45)	58 - 409 **217** (155)	78 – 382 **162** (90)
^{27}Al	240 – 543 **299** (56)	105 - 1414 **623** (404)	190 – 834 **471** (191)
^{39}K^{a}	568 – 1071 **717** (168)	221 - 997 **686** (199)	451 – 916 **663** (168)
^{43}Ca^{a}	55 – 86 **69** (8)	58- 105 **81** (14)	51- 98 **70** (13)
^{47}Ti	16 – 88 **43** (24)	12 - 70 **30** (23)	13 – 99 **49** (29)
^{51}V	0.8 – 5 **2** (1)	1.2 - 5 **2** (1)	1.2 – 3 **2** (1)
^{52}Cr	389 – 660 **546** (70)	437 - 821 **593** (135)	533 – 781 **603** (81)
^{53}Cr	510 – 836 **711** (78)	527 - 895 **763** (143)	623 – 870 **742** (76)
^{54}Fe	1060 – 5543 **1897** (1222)	263 - 1920 **1424** (676)	857 – 4170 **2037** (1425)
^{55}Mn	283 – 2507 **1449** (599)	882 - 2345 **1639** (512)	998 – 3017 **1980** (769)
^{57}Fe	1080 – 5514 **1903** (1215)	263 - 1890 **1423** (674)	853 – 4226 **2042** (1435)

^{a} Concentration (mg L^{-1})

Continuation Table

	Coast	**valley**	**Andes**
^{59}Co	0.1 – 10 **2** (3)	1 - 14 **4** (3)	1 – 6 **3** (1)
^{60}Ni	5 – 38 **15** (9)	7 - 31 **12** (6)	7 - 45 **20** (11)
^{63}Cu	22 – 138 **66** (37)	24 - 133 **67** (42)	16 - 300 **81** (76)
^{64}Zn	50 – 836 **175** (218)	130 - 575 **490** (27)	130 - 575 **241** (97)
^{65}Cu	27 – 138 **69** (38)	27 - 111 **81** (76)	16 - 300 **81** (76)
^{66}Zn	50 – 856 **179** (222)	332 - 785 **495** (141)	140 - 2495 **736** (798)
^{75}As	0.3 -1 **0.6** (0.1)	0.52 - 7 **1.0** (1.6)	0.4 – 0.9 **0.7** (0.2)
^{77}Se	1 – 7 **4** (2)	2 - 10 **5** (3)	2 – 8 **4** (2)
^{78} Se	1 – 6 **5** (1)	3 - 7 **4** (1)	3 – 6 **4** (1)
^{88}Sr	115 -1410 **744** (513)	109 - 486 **329** (154)	136 - 1260 **661** (373)
^{98}Mo	0.6 – 8 **2** (2)	0.3 - 6 **1** (2)	0.3 - 1 **1** (0)
^{107}Ag	0.8 – 5 **2** (1)	1.0 - 5 **2** (1)	0.7 - 4 **1** (1)
^{111}Cd	0.1 - 1.3 **0.2** (0.3)	0.1 - 1.0 **0.2** (0.2)	0.09 - 0.7 **0.3** (0.2)
^{118}Sn	1 – 9.0 **2.8** (2)	1 – 3.0 **2** (0.7)	0.3 – 3.0 **1.3** (0.8)
^{121}Sb	0.1 – 4.9 **0.5** (1)	0.1 – 1.0 **0.3** (1)	0.1 – 3.0 **0.6** (1)
^{138}Ba	32 – 730 **517** (198)	91 - 690 **282** (239)	90 - 413 **188** (102)
^{195}Pt	0.02 - 0.9 **0.1** (0.2)	0.01 - 1 **0.1** (0.3)	0.01 - 0.3 **0.1** (0.1)
^{202} Hg	0.4 - 1.3 **0.7** (0.2)	0.3 - 3.5 **0.9** (0.9)	0.3 - 1 **0.6** (0.2)
^{205}Tl	0.1 - 1.0 **0.3** (0.2)	0.24 - 0.6 **0.4** (0.1)	0.26 - 1.1 **0.6** (0.3)
^{208}Pb	2 – 58 **11** (17)	1 - 4 **2** (1)	2 - 34 **10** (12)

Table 4- 5. Summary of *red* wine samples composition by vineyard from Itata valley.

	Neira (*n* = 18)	**Entre Valles** (*n* = 4)	**Puertas** (*n* = 12)	**Piedras** (*n* = 12)	**Chillan** (*n* = 20)
^{7}Li	3.2 - 6.4	10 - 11	5 - 7	1 - 5	1 – 15
	4.4 (1.4)	**10** (0.15)	**6** (0.09)	**3** (2)	6 (5)
^{9}Be	0.2 -0.3	0.5 - 0.6	0.1 - 0.2	0.1 - 0.8	0.1 – 0.5
	0.2 (0.05)	**0.6** (0.02)	**0.2** (0.02)	**0.2** (0.2)	**0.2 (0.2)**
^{11}B	2995 - 4961	8367 - 8604	7388 – 8453	7081 – 222951	2936 – 16471
	3965 (735)	**8463** (101)	**7710** (352)	**14870** (6669)	**8592** (3243)
^{23}Na^{a}	3 - 6	61 - 63	12 - 16	4 - 7	4 – 24
	4 (0.8)	**61** (1)	**14** (2)	**6** (1)	12 (7)
^{24}Mg^{a}	62 - 141	89 - 92	136 - 152	101 – 335	77 – 280
	109 (33)	**90** (1)	**147** (6)	**224** (104)	**143 (42)**
^{26}Mg^{a}	70 - 141	102 - 105	131 – 150	116 - 294	88 – 268
	112 (29)	**103** (1)	**144** (8)	**213** (84)	**138 (36)**
^{31}P^{a}	45- 137	130 - 134	71 – 270	103 – 409	78 – 382
	80 (38)	**133** (2)	**94** (56)	**270** (145)	**174 (95)**
^{27}Al	240 - 370	260 - 270	280 - 543	400 - 1414	190 – 834
	305 (51)	**265** (6)	**313** (73)	**790** (317)	495 (166)
^{39}K^{a}	568 - 1071	836 – 856	554 -752	331 – 997	541 – 916
	780 (195)	**844** (9)	**622** (90)	**723** (220)	693 (169)
^{43}Ca^{a}	61 - 86	63 - 67	55 – 70	70 – 105	51 – 98
	74 (8)	**65** (2)	**64** (4)	**86 (13)**	**71 (14)**
^{47}Ti	230 - 796	799 - 865	240 – 885	122 – 700	126 – 880
	445 (223)	**821** (37)	**374** (188)	**330** (255)	**511 (321)**
^{51}V	0.8 - 4	0.9 - 1	2.1 – 3.0	1 -5	1 – 2
	2 (1)	**0.9** (0.1)	**2.3** (0.2)	**2** (1)	2 (0.4)
^{52}Cr	480 - 576	566 - 590	532 – 656	580 – 821	533 – 782
	541 (30)	**583** (10)	**611** (61)	**654** (97)	645 (77)
^{54}Fe	1060 - 2410	1200 - 1270	1095 - 1530	1680 – 1920	950 – 4170
	1617 (544)	1225 (33)	**1434** (124)	**1800** (84)	2270 (1455)
^{55}Mn	865 - 2507	935 - 931	1026 - 1646	882 – 2345	998 – 3017
	1790 (501)	**961** (24)	**1484** (237)	**1864** (371)	2044 (830)
^{57}Fe	1080 - 2420	1190 - 1280	1108- 1534	1680 – 1890	960 – 4226
	1624 (544)	**1233** (38)	**1447** (126)	**1798** (78)	2275 (1466)
^{59}Co	0.1 -1.6	1.6 – 1.9	1 – 3	1 – 14	1 – 6
	1 (1)	**1.8** (0.1)	**1.4** (0.9)	**5** (3)	3 (1)
^{60}Ni	5 - 14	9 - 10	17- 29	8 – 31	6 – 45
	9 (3)	**10** (0.4)	**20** (3)	**13** (6)	20 (13)

^{a} Concentration (mg L^{-1})

Continuation

	Neira (*n* = 18)	**Entre Valles** (*n* = 8)	**Puertas** (*n* = 4)	**Piedras** (*n* = 8)	**Chillan** (*n* = 16)
^{63}Cu	39 - 133	32 - 35	52 – 138	24 – 92	18 – 300
	80 (38)	**33** (1)	**70** (32)	45 (18)	94 (78)
^{64}Zn	50 - 190	70 - 90	69 – 145	380 – 762	130 – 575
	122 (50)	**80** (8)	**81** (22)	535 (123)	241 (107)
^{65}Cu	40 - 130	30 - 30	60 – 138	30 – 111	20 – 238
	79 (38)	**30** (0)	**80** (34)	57 (28)	89 (71)
^{66}Zn	50 - 190	80 - 90	70 – 150	380 – 785	140 – 2495
	126 (51)	**83** (5)	**84** (22)	545 (128)	834 (842)
^{75}As	0.3 - 0.8	0.65 – 0.7	0.43 - 0.99	1 – 7	0.4 – 0.9
	0.5 (0.2)	**0.68** (0.02)	**0.6** (0.1)	1 (2)	0.6 (1)
^{77}Se	1 - 7	3.6 – 7.0	2 – 6	2 – 10	1 – 8
	5 (1)	**6** (2)	**3** (2)	6 (3)	4 (2)
^{78} Se	5 - 6	5.4 – 5.5	3 – 6	3 – 7	3 – 6
	5.5 (0)	**5.4** (0.04)	**4** (1)	5 (1)	5 (1)
^{88}Sr	670 - 1410	1090 - 1130	115 – 235	109 – 485	136 – 1260
	1136 (326)	**1113** (17)	**175** (51)	278 (145)	656 (410)
^{98}Mo	1 - 8	1.06 – 1.14	1 – 7	0.3 – 0.6	0.3 – 1
	2 (2)	**1** (0.04)	**3** (2)	0.4 (0.1)	0.6 (0.2)
^{107}Ag	1.3 - 4.6	1.3 – 1.4	0.8 – 5.2	1 – 1.5	0.9 – 3.7
	1.8 (0.8)	**1.3** (0.05)	**2.4** (1.5)	1.2 (0.2)	1.5 (0.7)
^{111}Cd	0.1 - 0.2	0.09 - 0.12	0.2 - 0.5	0.1 – 1.0	0.1 – 0.7
	0.1 (0.04)	**0.11** (0.1)	**0.2** (0.1)	0.2 (0.2)	0.3 (0.2)
^{118}Sn	1.8 – 2.2	1.8 – 2.0	0.7 – 2.3	0.3 – 1.5	0.5 – 1.6
	1.0 (0.5)	1.9 (0.1)	**1.5** (0.5)	1 (0.8)	1 (2)
^{121}Sb	0.2 – 1.9	0.16 – 0.18	0.1 – 0.8	0.1 – 0.2	0.5 – 0.6
	0.6 (1.1)	**0.17** (0.5)	**0.4** (0.3)	0.1 (0.1)	0.3 (0.2)
^{138}Ba	560 - 730	380 - 480	121 – 604	91 – 690	90 - 413
	267 (66)	**450** (48)	**536** (135)	320 (268)	205 (104)
^{195}Pt	0.02-0.5	0.02 - 0.03	0.2 - 0.9	0.01 – 0.09	0.01 – 0.3
	0.1 (0.1)	**0.023** (0.005)	**0.4** (0.2)	0.03 (0.03)	0.1 (0.1)
^{202} Hg	0.4 - 0.9	0.4 - 0.5	0.4 – 1.3	0.3 – 0.6	0.3 – 0.7
	0.6 (0.1)	**0.48** (0.065)	**0.8** (0.3)	0.4 (0.1)	0.5 (0.1)
^{205}Tl	0.3 - 0.4	0.141-0.170	0.2 – 1.0	0.2 – 0.6	0.3 – 1.0
	0.3 (0.1)	**0.153** (0.013)	**0.3** (0.2)	0.4 (0.1)	0.5 (0.3)
^{208}Pb	2 - 5	0.14 – 0.14	6 – 33	1 – 3	2 – 34
	3 (1)	0.14 (0)	**11** (8)	2 (1)	11 (13)

[a] Concentration (mg L^{-1}).

Table 4- 6. Summary of *white* wine samples composition by vineyard from Itata valley. Ranges, means, and standard deviations.

	Puerta del Itata ($n = 6$)	**Piedras del Encanto** ($n = 4$)	**Chillan** ($n = 4$)
^{7}Li	1.56 – 1.7	5.2 - 5.4	1.6- 1.7
	1.6 (0.1)	**5.3** (0.1)	**1.6** (0.04)
^{9}Be	0.11 – 0.12	0.13 - 0.17	0.07 - 0.09
	0.11 (0.01)	**0.15** (0.02)	**0.08** (0.01)
^{11}B	422 - 4602	247 - 521	5435 - 5588
	4390 (186)	**248** (22)	**5519** (77)
^{23}Na^{a}	9.0 - 10	8.96 -9.77	10.9 - 11.5
	9.5 (0.5)	**9.39** (0.39)	**11** (0.3)
^{24}Mg^{a}	70 - 78	88 - 90	115 - 118
	73 (4.3)	**89** (1.2)	**116** (1.3)
^{26}Mg^{a}	66 - 74	83 - 85	108 - 110
	69 (4)	**84** (0.8)	**109** (1)
^{31}P^{a}	48 - 53	58.02 - 59.89	101 - 103
	50 (3)	**59.17** (0.89)	**102** (1)
^{27}Al	252 - 273	105 - 162	340 - 353
	263 (11)	**123** (26)	**347** (5)
^{39}K^{a}	591 - 595	564 - 599	496 - 523
	592 (1.9)	**576** (16)	**511** (14)
^{43}Ca^{a}	70 - 74	58 - 75	60.2 - 70.1
	72 (2)	**67** (7.2)	**66** (4.1)
^{47}Ti	159 - 182	195 - 210	339 - 372
	171 (10)	**203** (6)	**358** (16)
^{51}V	3.8 - 4.8	3.3 - 3.8	2.69 - 2.74
	4.5 (0.2)	**3.5** (0.2)	**2.71** (0.03)
^{52}Cr	390 - 420	441 - 458	591 - 622
	404 (14)	**451** (7)	**606** (13)
^{54}Fe	4974 – 5543	263- 314	857 - 886
	5215 (275)	**296** (23)	**874** (13)
^{55}Mn	282 - 311	957 - 979	1636 - 1691
	295 (14)	**965** (10)	**1658 (**24)
^{57}Fe	4960 - 5513	263 - 311	853 – 888
	5198 (274)	**298** (23)	**875** (15)

^{a} Concentration (mg L^{-1})

Continuation Table

	Puerta	**Piedras**	**Chillan**
^{59}Co	9 - 10	2.1 - 2.6	3.2 - 3.3
	9.5 (0.5)	**2.3** (0.2)	**3.28** (0.05)
^{60}Ni	34 - 38	6.8 – 7.7	16.8 – 17.1
	36 (2)	**7.5** (0.5)	**16.9** (0.1)
^{63}Cu	22 – 24	131 – 132	15.8 – 20
	23 (1)	**132** (0.7)	**17.5** (2)
^{64}Zn	755 - 836	322 - 353	233 - 241
	789 (40)	**337** (15)	**238** (3.5)
^{65}Cu	26 - 27	26 - 26	19 - 24
	26 (0)	**26** (0)	**21** (2.5)
^{66}Zn	0.6 – 0.7	0.5 - 0.9	0.8 - 0.9
	0.6 (0)	**0.6** (0.2)	**0.85** (0.08)
^{75}As	768 - 856	332 - 364	236 - 246
	806 (44)	347 (17)	242 (4.6)
^{77}Se	3.4 – 4.2	2.0 -5.4	2.2 - 2.7
	3.8 (0.4)	**3.6** (1.5)	**2.5** (0.2)
^{78} Se	3.2 - 3.4	3.3 – 4.0	2.9 - 3.4
	3.3 (0.1)	**3.6** (0.3)	**3.2** (0.2)
^{88}Sr	304 - 341	478 - 486	676 - 696
	322 (18)	**481** (3.3)	**681** (9.7)
^{98}Mo	1.7 – 1.8	1.1 - 6	0.8 - 0.9
	1.7 (0.1)	**3.4** (2.3)	**0.88** (0.05)
^{107}Ag	1 – 1.5	2.0 – 4.5	0.7 - 1.6
	1.2 (0.2)	**2.8** (1.2)	**1.1** (0.4)
^{111}Cd	0.8 - 1.3	0.1 - 0.3	0.12- 0.15
	1 (0.2)	**0.18** (0.1)	**0.14** (0.01)
^{118}Sn	1.9 – 2.3	0.4 – 2.0	2.0 - 3.1
	2 (0.1)	**1** (15)	**3** (0.3)
^{121}Sb	0.9 – 1.0	1.3 – 3.3	0.7 - 0.9
	1 (0)	**2.4** (0.1)	**0.8** (0.5)
^{138}Ba	32 - 34	167 -169	103 - 105
	23 (1.2)	**168** (1.2)	**104** (1)
^{195}Pt	0.04 - 0.1	0.22 - 1.08	0.05 - 0.07
	0.1 (0.06)	**0.5** (0.4)	**0.06** (0.01)
^{202} Hg	0.97 - 1.04	1.6 - 3.5	0.8 – 1.0
	1.01 (0.03)	**2.3** (0.9)	**0.9** (0.1)
^{205}Tl	0.11 - 0.12	0.5 - 0.6	1.02 - 1.05
	0.12 (0.01)	**0.5** (0.02)	**1.03** (0.01)
^{208}Pb	54 - 58	3.5 - 3.8	3.37 - 3.52
	56 (2.2)	**3.6** (0.1)	**3.42**(0.07)

4.4.2. Multivariate decomposition methods

Principal Components Analysis (PCA), is a nonsupervised method which is useful to resolve multivariate data by reducing the dimensionality into some correlated variables, called the latent variables (principal components) [32].

The lineal combination of the variables generates projections of the data set corresponding a matrix of scores and loadings. The interpretation of these matrices reflects the natural tendencies, the synergistic or antagonistic relations between variables and quantities. The processing of the data in matrices with discrete value is auto-scaled to avoid the difference in the concentration of the original variables.

The exploration in the original set with 83 samples and 27 variables showed six optimal factors; the first three factors show 55% in the cumulative variance. Thus the contribution of more variables to improve the grouping is required. Several conclusions were obtained from the plot of scores. The separation between groups known a priori as coast, valley, and Andes are appreciable, also, the samples of Csl were put together on an independent cluster, and the white wine is not grouped with the samples of red wine, and significative distance differs between these two groups.

The relation with the groupings in score plot and original variable through the loading plot were analyzed .The samples that come from the coast are oriented to the first and second factor for Ca, Mn, Cr, Fe, Sr and Be. In the intermediate valley, the principal variables were Na, B, Sb, and Ti. Moreover, the most important element for Andes samples was Zn, Al, Mg, P, and Cr. On the other hand, The Cmn wine samples from Andes, was grouped independently of red wines and white wines. For separation of these samples, the implicated variables were Pb, Cu, Ni, and Tl.

Parallel, the Csl group are conformed by samples from the different zones and Li, K, Se, and Ba are their general descriptors.

Independent studies of Csl group were allowed between by geographical area. The accumulate variance was 91%, and the original variable for Csl coast was Tl, Ag, Ca, Sb, Ba, Mo, Pt, and Pb. Csl valley Fe, Al, Ni, Mn, Co, Be, Se, Sr, and K; Csl Andes, Cr, Li, P, Na, Ti, Zn and Mg. For white wines, the clustering of Ch attributed to the concentration of Fe, Ni, Pb, Zn, B, Al, K and Ca. For SB, Na, Cr, P and Ti, and Finally for M, Li, Sb, Be, Ba, Mn, Sr, Tl, and Mg. Corresponding descriptors for grapes of each vineyard according to geographical area it is possible to identify (see STable 4- 9).

The appropriated soft independent modeling by class analogy (SIMCA) and Linear discriminant analysis (LDA) were used in the supervised modeling, Figure 4- 1. To evaluate the reduction of variables, indices of modeling power carried in SIMCA were used. Subsequently, the variables of prediction were Al, Pb, Na, Tl, Mo, Ca, Cr, Se, Ni, Zn and B. The stability and robustness of the models were checked by the leave-one-out (LOO) method. For this instance, two sets of data are prepared, one to create a prediction model and another to validate, the percentages calculated are in Table 4- 7.

Hence, the predictive values found were 100% geographical designation. The PCA analysis and the statistical indices suggest the most propriety of wine for geographical differentiation. Also, the zones of classification were selected in the relation to the know denomination, coast, intermediate valley and Andes. Some grape varieties have been a high impact as mentioned earlier.

Table 4- 7. Results of the models found for the geographical origin classification of Itata valley by SIMCA and LDA with the percentage of cases correctly classified in data set and external set.

	SIMCA		LDA	
categories	**data set**	**External set**	**data set**	**External set**
Coast	98%	83%	91%	84%
Valley	97%	67%	90%	81%
Andes	93%	84%	94%	82%

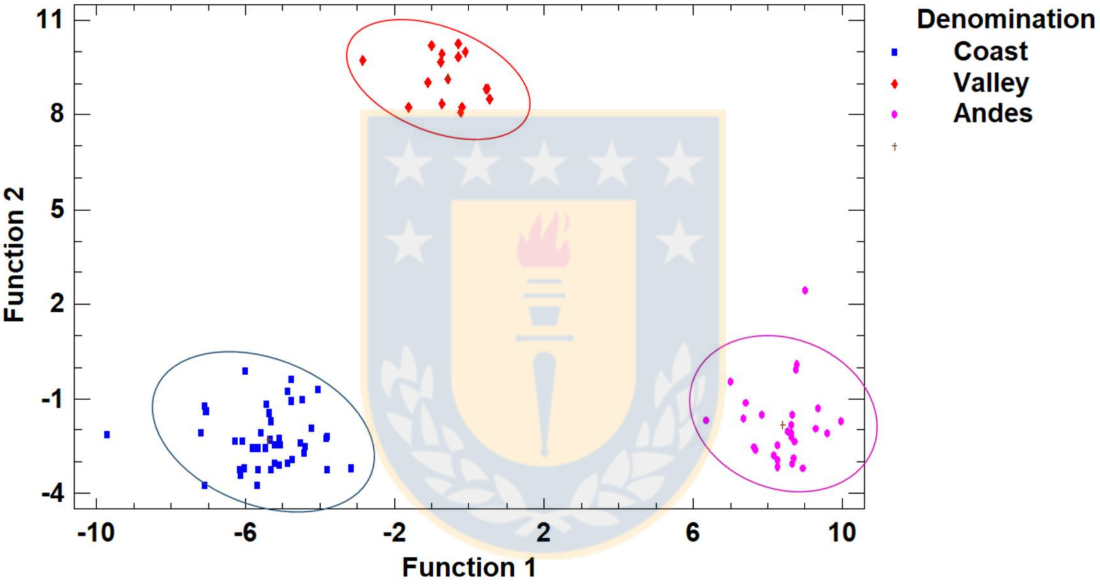

Figure 4- 1. Graph of first and second functions for the linear discriminant analysis (LDA). For wine classification according to their geographical denomination as coast, valley or andes.

4.4.3. Trace metal study using size exclusion chromatography/anion exchange chromatography-inductively coupled plasma-mass spectrometry

To investigate the compounds bound to elements in wines, analyzes were carried out by size exclusion chromatography (SEC). In the optimization methodology, the choice of the appropriate column to separate the group of masses corresponding to low molecular weight compounds between 100 and 7000 Da. Besides, the appropriate concentration of ionic strength and value of pH of mobile phase for red and white wine was determined. The result is showed in the SFigure 4- 1 and SFigure 4- 2.

The calibration with PEOs the retention time (Rt) and the logarithm of the molecular weight (Log(Mw)) data were adjusted to a lineal model, and the good lineal relationship was achieved (regression coefficient, R^2, of 0.9506 for a calibration equation was Log (Mw) = $-0.115x + 5.109$. The exclusion volume (V_0) was determined using Blue dextran, which implies a retention time of 9.26 min; while the permeation volume (Vp) was fixed by injecting Milli-Q water, which corresponds to a retention time of 25min (see SFigure 4- 3 and STable 4- 10).

The allowed molecular weight distribution profiles are then in the range of 200 to 7000 Da. However, of the peaks observed in the chromatogram SEC was calculated that for these samples the molecular weight range is between 200 and 2000 Da. The samples injected corresponding to Cabernet Sauvignon, Pinot noir, Cinsault, Carmenere, Malbec, Chardonnay, Muscat of Alexandria and Sauvignon blanc for red wine and white respectively. The profiles for each wine from the chromatograms be able to be seen in SFigure 4- 4. For all cases, analysis was performed in triplicate, and different blanks were also analyzed for contamination control.

The fractions were collected and weighed in plastic tubes and analyzed by ICP-MS. The LOD and LOQ of were determinate (**¡Error! No se encuentra el origen de la referencia.**). The main fractions were selected according to the concentration of elementals found according to the values higher than the LOD. The result is in Figure 4- 1 was the data with simultaneous spectrum along with the complete chromatogram of the wines are overlapped. From these results, the relation between the element and the molecular weights are according to the retention time (STable 4- 11). Elements such as B, Cu, Ni, Rb, and Ti were found to be bounded to compounds of molecular weights within the 200-2000 Da range. The SEC fractions (2 - 4 mL) were diluted to 6 mL with the HNO_3, and the same internal standards (at the same concentrations listed above) were used for calibration. The elements found in the fractions were 12 among which were found: Li, B, ^{53}Cr, Ti, Mn, ^{65}Cu, Mo, Ba, V, Ni, and Sb, showed in STable 4- 9.

4.4.4. Screening wine samples by anion exchange chromatography

This SEC fraction was further characterized by AEC (use of PR-X100 column) and three compounds (UV detection). F1, F2, and F3 (retention times of 2.6, 3.2. and 3.8 min) were observed. AEC-ICP-MS showed the presence of B-, Rb-, and Li-LMWC complexes. B was found to be bounded to LMWC in fraction F2; whereas, Li was associated to fraction F1. However other elements such as Rb and Mn, which were associated with the investigated SEC fraction. Were not observed after AEC-ICP-MS analysis. These finding could imply high lability of Rb- and Mn-LMWC complexes.

Table 4- 8. LOD and LOQ for the white and red wine in SEC fractions.

	Red		**White**	
μg L^{-1}	**LOD**	**LOQ**	**LOD**	**LOQ**
^{7}Li	0.06	0.2	0.04	0.1
^{11}B	0.2	0.5	1	2
^{26}Mg	0.7	2.2	0.2	0.8
^{27}Al	0.3	0.9	0.2	0.5
^{52}Cr	0.1	0.4	0.2	0.7
^{55}Mn	0.6	1.9	0.1	0.5
^{51}V	0.01	0.04	0	0.1
^{57}Fe	3.9	12.8	1	3.2
^{60}Ni	0.2	0.7	0.5	1.5
^{65}Cu	0.7	2.5	0.6	2
^{66}Zn	1.6	5.5	0.6	1.9
^{78}Se	0.05	0.16	0.1	0.2
^{88}Sr	0	0.1	0	0
^{98}Mo	0.1	0.2	0.2	0.6
^{107}Ag	0.2	0.6	0.1	0.2
^{118}Sn	0.2	0.6	0.1	0.4
^{121}Sb	0.1	0.3	0.4	1.4
^{138}Ba	6.7	22.5	1	3.5
^{202}Hg	0.03	0.09	0.1	0.3

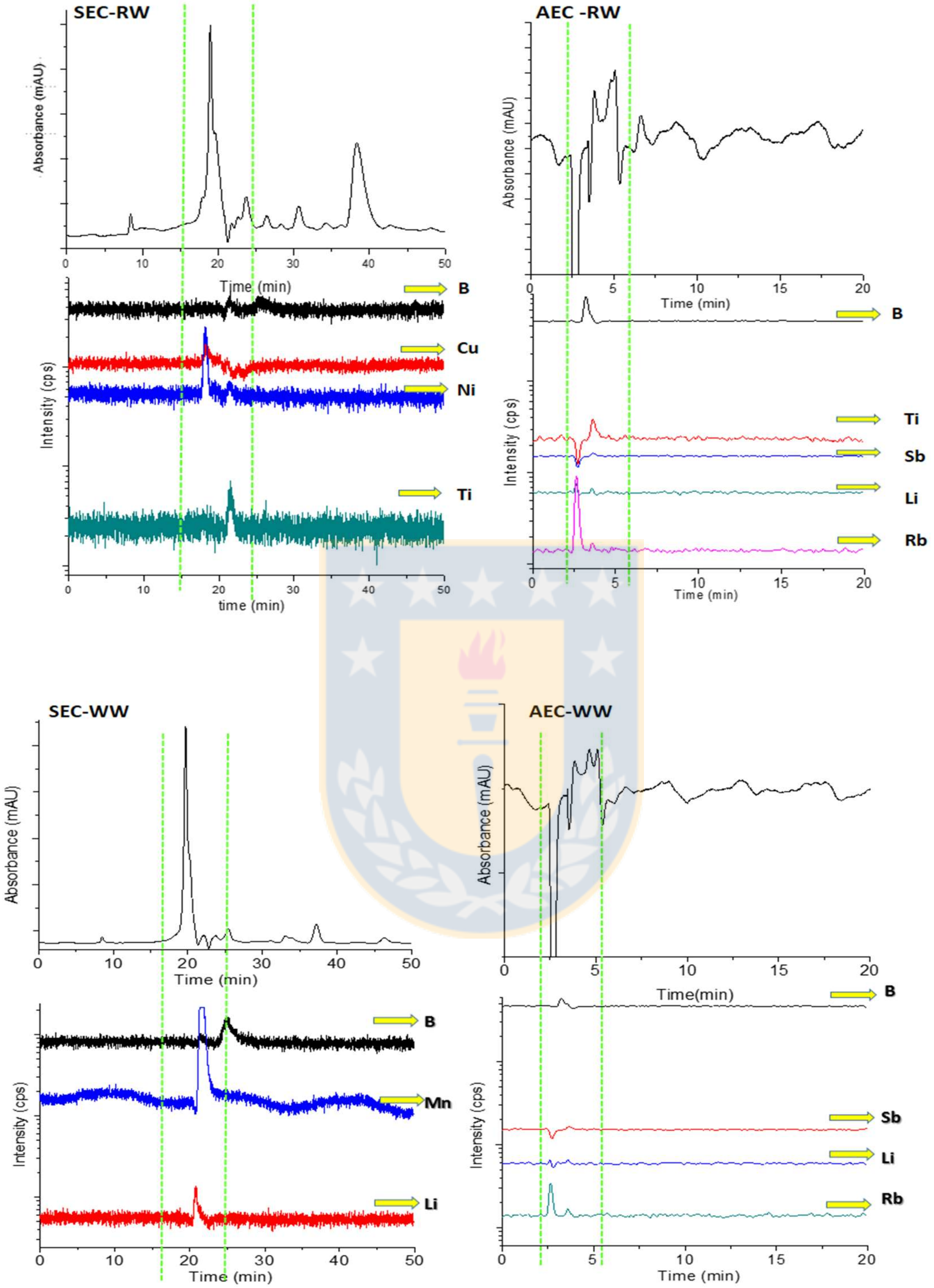

Figure 4- 2. SEC/AEC chromatograms (UV and ICP-MS detection) of red wine and white wine samples pinot noir and chardonnay (from Neira and Puertas del Itata vineyard, denomination coast, and valley respectively)

Table 4- 9. Elements deterrminated in SEC fractions.

Red			
Fraction SEC	*µg L^{-1}*	*Elements*	*tr*
24Cmn - F2	34	B	17 - 21.3
24Cmn - F3	0.2	Li	22.5 - 24.3
	5	Sn	
	4	Sb	
8CS - F3	29	B	20 - 21.3
8CS - F6	0.4	Li	24.5 - 25.5
8CS - F7	0.3	Li	27.8 - 30.3
16Csl - F5	0.5	Li	22.9 - 24.3
	4	^{55}Mn	
	13	^{60}Ni	
29Cmn - F2	3	Sn	22.5 - 24.3
29Cmn - F7	0.2	Li	13.1 - 15
05Pn - F2	0.2	Li	13.1 - 14.9
05Pn - F4	22	^{65}Cu	18.9 - 19.9
37Mlb - F1	3	Sn	17.9 - 21.4
37Mlb - F2	2	Sb	22.5 - 24.3
White			
Fraction SEC	*µg L^{-1}*	*Elements*	*tr*
32M - F2	0.3	Li	10 - 11
	3.1	^{53}Cr	10 - 11
	0.2	V	10 - 11
	0.8	Mn	10 - 11
	1.8	Ni	10 - 11
	1.5	Sn	10 - 11
32M - F3	6	^{65}Cu	16 - 21.3
32M - F4	135	B	24.06 - 26.05
26SB - F2	3	^{53}Cr	16.9 - 21
	1.8	^{55}Mn	16.9 - 22
26SB - F3	9	Ni	22.8 - 24.5
26SB - F4	42	B	24.5 - 26.5
21CNN - F3	2	Ni	17.20 - 21.3
21CNN - F4	9	^{55}Mn	24.6 - 26.2

Cmn: Carmenere, CS: Cabernet Sauvignon, Csl: Cinsault, Pn: Pinot noir, Mlb: Malbec. M: Muscat of Alexandria, SB: Sauvignon Blanc, CNN: Chardonnay, tr: retention time.

4.5. Conclusions

The Inductively coupled plasma mass spectrometry analysis and different chemometric approaches, the discrimination of different red and white wine samples from Itata valley can be achieved. The results showed a high percentage of classification when the samples of red and white wine were studied separately, is the group of the white wine the one that presented the highest classification percentages and significant distances between categories. Classification according to the denotation of the coast, valley, and Andes generates high prediction results. While through the study of metal complexes with two-dimensional chromatography and ICP-MS, was able to relate fractions in B wines. Opening the possibility of developing speciation studies through metalomics approach.

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Supplementary Material

STable 4- 1. Operating ICP-MS conditions.

General		
	Radiofrequency power/W	1400
	Sample uptake rate/r.p.m.	3
	Stabilization delay/s	35
	Number of replicates	3
Nebulizer type	Beat impact (cooled spray chamber)	
Gas flows/L min^{-1}	Plasma	13
	Auxiliary	0.8
	Nebulizer	0.9
Ion optics/V		
	Extraction	-125
	Lens 1	-1000
	Lens 2	-80
	Lens 3	-195.3
	Hexapole Bias	-4
	Pole Bias	0.2
	D1	-40.8
	D2	-140
	Hexapole Bias	-4
	Pole Bias	0.2
Torch alignment/mm		
	Horizontal	80
	Vertical	405
	Sampling death	150

Continuation

Mass-to-ratio	
	^{7}Li, ^{9}Bo, ^{11}B, ^{23}Na, ^{24}Mg, ^{26}Mg, ^{27}Al, ^{29}Si, ^{31}P, ^{39}K, ^{43}Ca, ^{47}Ti, ^{51}V, ^{52}Cr, ^{53}Cr, ^{54}Fe, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{65}Cu, ^{66}Zn, ^{75}As, ^{77}Se, ^{78}Se, ^{88}Sr, ^{98}Mo, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{138}Ba, ^{195}Pt, ^{202}Hg and ^{208}Pb.
	Internal standards
	^{54}Sc, ^{72}Ge, ^{89}Y, ^{115}In

STable 4- 2. SEC/AEC-UV/ICP-MS operating conditions.

SEC	TSK-G4000 SWXL (30 cm × 8 mm i.d.) coupled to a TSK-gel SW glass guard column (10 cm × 8 mm i.d.)	
	Injection volume (µL)	50
	Column temperature (°C)	25
	Mobile phases flow rate/mL min-1	1
Mobile phase	25 mM/25 mM ammonium sulphate/diammonium hydrogen phosphate, pH 6.5	
	UV detection (nm)	205
AEC	PRP-X100 (250 mm × 4.1 mm i.d.) to a PRP-X100 guard column (25 × 2.3 mm i.d.)	
	Injection volume (µL)	50
	Column temperature (°C)	25
	Mobile phases flow rate/mL min-1	1
Mobile phase	25 mM/25 mM ammonium sulphate/diammonium hydrogen phosphate, pH 6.5	
	UV detection (nm)	205
ICP-MS		
	Radiofrequency power/W	1400
	Peristaltic pump speed/r.p.m.	2.5
Nebulizer type	Beat impact (cooled spray chamber)	
Gas flows/L min-1	Plasma	13
	Auxiliary	0.8
	Nebulizer	0.9
Ion optics/V		
	Extraction	-125

Continuation

	Lens 1	-1000
	Lens 2	-80
	Lens 3	-195
	Focus	11
	D1	-40.8
	D2	-140
	Pole Bias	0.2
	Hexapole Bias	-4
Torch alignment/mm		
	Horizontal	80
	Vertical	405
	Sampling death	150
Mass-to-ratio		
	^{7}Li, ^{11}B, ^{47}Ti, ^{51}V ,^{53}Cr, ^{55}Mn, ^{60}Ni, ^{65}Cu, ^{98}Mo, ^{138}Ba, and ^{121}Sb.	

STable 4- 3. Metal average levels reported in the literature for wine samples.

Average levels / µg L^{-1}							
	OIV. O, 2016	Thiel, 2004	Fabani, 2010	Geana, 2013	Betlin, 2011	Mercosur regulation	Azcarate, 2015
Li	10 - 50	17.6			3-14		941
Be		1.27		7.29			17
V	3 - 200			47.61	21-281		358
Mn	500 - 1500		0.8	806	430-3270		1778
Co		4.65		4.35	2-12		24
Ni		24.9					276
Cu							186
Ge							6.3
As		7.1			4-79		20
Rb	500 - 1200	349	890.11		1110-5935		531
Sr	300 - 1000		540.55				556
Mo		11.3			2.2-38		10
Cd	1 - 40	0.41			< 0.01-6		7.0 x 10 $^{-1}$
Ba							29
Tl							6.5 x 10 $^{-1}$
Pb	10 - 200			37.97	2.5-28		3
Bi							3.0x 10 $^{-1}$
Al	250 - 5000						
B	10000 - 40000					Max 80000 as boric acid	

Average levels / $\mu g\ L^{-1}$	OIV. O, 2016	Thiel, 2004	Fabani, 2010	Geana, 2013	Betlin, 2011	Mercosur regulation	Azcarate, 2015
Br	200 - 2500						
Co	2 - 50						
Cu	100 - 2000			500.57	73-346		
Fe	800 - 5000		3.7				
Mg	50 – 300[a]		70		99-124[a]		
Ni	10 - 200			55.15	3-28		
Na	5 – 30[a]						
Zn	30 – 1000[a]		0.3	433.95	140-1359		
Ti	40.4				70-186		
Sb		1.65					
Cs		4.00					
La		0.70					
Ce		1.36					
U		0.55			< 0.02-1.2		
K			488		899-1395[a]		
Na			36				
Ca			50		28-73		
Cr			0.8	255	3-61		
Ag				7.01	< 0.05-1		
Sn					< 0.07-1		
P							

STable 4- 4. The F - Reason in agreement of the ANOVA result, 95% confidence level, $p<0.05$. The significant difference between elements according to the type of wine, white wine samples by vineyard, red wine samples by vineyard and geographical denomination.

Type		***White vineyard***		***Red vineyard***		***denomination***	
Elements	F-Reason	Elements	F-Reason	Elements	F-Reason	Elements	F-Reason
^{53}Cr	36	^{208}Pb	5062	^{23}Na	59	^{66}Zn	7.4
^{202}Hg	17	^{138}Ba	3842	^{64}Zn	19	^{11}B	6.4
^{51}V	12	^{63}Cu	2621	^{88}Sr	9	^{205}Tl	5.9
^{65}Cu	9	^{55}Mn	1643	^{138}Ba	8	^{64}Zn	5.9
^{138}Ba	7	^{7}Li	769	^{53}Cr	7	^{31}P	4.1
^{52}Cr	7	^{11}B	460	^{195}Pt	6	^{138}Ba	3.6
^{78}Se	6	^{54}Fe	293	^{27}Al	6	^{121}Sb	3.1
^{55}Mn	6	^{57}Fe	292	^{11}B	5	^{59}Co	2.6
^{59}Co	5	^{31}P	258	^{26}Mg	3	^{27}Al	2.5
^{208}Pb	4	^{88}Sr	229	^{31}P	3	^{52}Cr	2.3
^{26}Mg	4	^{60}Ni	157	^{59}Co	3	^{24}Mg	2.0
^{9}Be	3	^{64}Zn	142	^{24}Mg	3	^{98}Mo	2.0
^{111}Cd	3	^{66}Zn	126	^{52}Cr	3	^{208}Pb	2.0
^{39}K	3	^{47}Ti	86	^{66}Zn	2	^{26}Mg	1.9
^{11}B	3	^{59}Co	84	^{43}Ca	2	^{43}Ca	1.5
^{47}Ti	3	^{26}Mg	73	^{9}Be	2	^{55}Mn	1.3
^{24}Mg	2	^{24}Mg	72	^{60}Ni	2	^{60}Ni	1.1
^{31}P	2	^{52}Cr	71	^{7}Li	2		
^{98}Mo	2	^{27}Al	47	^{205}Tl	2		
^{7}Li	2	^{53}Cr	17	^{208}Pb	1		
^{205}Tl	2	^{39}K	12	^{55}Mn	1		
^{77}Se	2	^{65}Cu	5	^{107}Ag	1		
^{27}Al	1	^{23}Na	3	^{47}Ti	1		
		^{51}V	3	^{78}Se	1		

Continuation

Type		***White vineyard***		***Red vineyard***		***denomination***	
Elements	*F*-Reason	Elements	*F*-Reason	Elements	*F*-Reason	Elements	*F*-Reason
		^{78}Se	2	^{77}Se	1		
		^{121}Sb	2	^{54}Fe	1		
		^{107}Ag	1	^{57}Fe	1		
		^{75}As	1				
		^{118}Sn	1				

STable 4- 5. The *F* - Reason in agreement of the ANOVA result, 95% confidence level, p<0.05. The significant difference between elements according to grape for white and red wine and the group with both.

grape - White wine		***grape – Red wine***		***All wine grape varieties***	
Elements	*F*-Reason	Elements	*F*-Reason	Elements	*F*-Reason
^{138}Ba	3749	^{27}Al	13	^{52}Cr	127
^{63}Cu	2557	^{88}Sr	10	^{11}B	34
^{55}Mn	1603	^{64}Zn	10	^{27}Al	13
^{7}Li	750	^{26}Mg	9	^{26}Mg	11
^{208}Pb	494	^{24}Mg	9	^{24}Mg	9
^{11}B	432	^{195}Pt	7	^{202}Hg	9
^{54}Fe	285	^{118}Sn	6	^{88}Sr	9
^{57}Fe	285	^{7}Li	5	^{78}Se	8
^{31}P	251	^{66}Zn	5	^{66}Zn	8
^{88}Sr	223	^{60}Ni	4	^{64}Zn	7
^{60}Ni	154	^{11}B	4	^{7}Li	6
^{64}Zn	138	^{31a}P	4	^{60}Ni	6
^{66}Zn	123	^{39}K	3	^{54}Fe	6
^{47}Ti	84	^{202}Hg	3	^{208}Pb	6
^{59}Co	82	^{208}Pb	3	^{57}Fe	6
^{26}Mg	71	^{51}V	3	^{205}Tl	5
^{24}Mg	70	^{43}Ca	2	^{39}K	5
^{52}Cr	69	^{47}Ti	2	^{31}P	4
^{27}Al	45	^{107}Ag	2	^{195}Pt	4
^{39}K	12	^{9}Be	2	^{47}Ti	3
^{111}Cd	11	^{52}Cr	2	^{59}Co	3
^{65}Cu	5	^{63}Cu	2	^{55}Mn	3
^{23}Na	3	^{205}Tl	1	^{107}Ag	3
^{51}V	3	^{59}Co	1	^{98}Mo	2

Continuation

grape - White wine		***grape – Red wine***		***All wine grape varieties***	
Elements	*F*-Reason	Elements	*F*-Reason	Elements	*F*-Reason
^{202}Hg	2	^{138}Ba	1	^{77}Se	2
^{78}Se	2	^{78}Se	1	^{138}Ba	2
^{121}Sb	2	^{54}Fe	1	^{9}Be	2
^{107}Ag	1	^{65}Cu	1	^{121}Sb	2
^{75}As	1	^{57}Fe	1	^{43}Ca	2
^{118}Sn	1	^{75}As	1	^{63}Cu	2
		^{121}Sb	1	^{65}Cu	1
		^{77}Se	1		

STable 4- 6. Concentration of elements in white wine samples according to different grape varieties. The composition obtained with ICPMS.

Elements	**Ch**	**M**	**SB**
^{7}Li	2 ± 0.07	5 ± 0.07	2 ± 0.04
^{9}Be	0.1 ± 0.01	0.1 ± 0.02	0.08 ± 0.01
^{11}B	4589 ± 247	2482 ± 191	5572 ± 87
^{23a}Na	10 ± 1	9 ± 0.39	11 ± 0.3
^{24a}Mg	74 ± 4.3	89 ± 1.25	116 ± 1.3
^{26a}Mg	69 ± 3.9	85 ± 0.84	109 ± 1.0
^{27}Al	263 ± 11	123 ± 26	348 ± 6
^{31a}P	50 ± 2.7	59 ± 1	102 ± 1
^{39a}K	593 ± 1.9	576 ± 16	512 ± 14
^{43a}Ca	72 ± 2.1	67 ± 7	66 ± 4
^{47}Ti	187 ± 10	195 ± 24	368 ± 33
^{51}V	5 ± 0.2	3 ± 0.2	3 ± 0.03
^{52}Cr	400 ± 14	415 ± 17	671 ± 22
^{54}Fe	5216 ± 275	296 ± 23	875 ± 13
^{55}Mn	296 ± 14	965 ± 10	1659 ± 24
^{57}Fe	5199 ± 274	298 ± 23	876 ± 15
^{59}Co	9 ± 0.5	2.3 ± 0.2	3 ± 0.1
^{60}Ni	36 ± 1.9	7.5 ± 0.5	17 ± 0.1
^{63}Cu	23 ± 1.0	132 ± 0.7	18 ± 2
^{64}Zn	790 ± 40	337 ± 16	238 ± 3
^{65}Cu	27 ± 0	27 ± 0.0	21 ± 3
^{66}Zn	806 ± 44	347 ± 17	243 ± 5
^{75}As	0.6 ± 0.04	0.6 ± 0.2	0.9 ± 0.1
^{77}Se	3.8 ± 0.4	3.6 ± 1	2.5 ± 0.2

Ch (Chardonnay), **M** (Muscat), **SB** (Sauvignon Blanc). ^{a} Concetration (mg L^{-1}).

Continuation

Elements	**Ch**	**M**	**SB**
^{78}Se	3.3 ± 0.1	3.6 ± 0.3	3.2 ± 0.2
^{88}Sr	322 ± 18	482 ± 3	682 ± 10
^{98}Mo	1.7 ± 0.1	3.4 ± 2	0.9 ± 0.0
^{107}Ag	1.2 ± 0.2	2.8 ± 1.2	1.1 ± 0.4
^{111}Cd	1.0 ± 0.2	0.2 ± 0.1	0.1 ± 0.05
^{118}Sn	2.1 ± 0.1	18 ± 15	2.7 ± 0.3
^{121}Sb	1.0 ± 0.0	2 ± 1.0	0.8 ± 0.1
^{138}Ba	33 ± 1.2	168 ± 1.2	105 ± 1
^{195}Pt	0.1 ± 0.01	0.5 ± 0.4	0.1 ± 0.01
^{202}Hg	1.0 ± 0.03	2.3 ± 0.9	0.9 ± 0.1
^{205}Tl	0.1 ± 0.01	0.5 ± 0.02	1.0 ± 0.01
^{208}Pb	56 ± 2	3.6 ± 0.1	3.4 ± 0.1

Ch (Chardonnay), **M** (Muscat), **SB** (Sauvignon Blanc). ^{a} Concetration (mg L^{-1}).

STable 4- 7. The concentration of elements in red wine samples according to different grape varieties. The composition obtained with ICP - MS.

Elements	Csl	Pn	CS	PB	P	Mlb	Cmn
Li 7	9 ± 4	-[a]	-[a]	-[a]	-[a]	-[a]	-[a]
Be 9	0.2 ± 0.04	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.02	0.1 ± 0.03	0.2 ± 0.2	0 ± 0.1
B11	3789 ± 50	5905 ± 40	8218 ± 31	780 ± 45	787 ± 37	1987 ± 59	8294 ± 97
Na23 [a]	9 ± 5	4 ± 1	18 ± 24	13 ± 0.5	15 ± 2	6 ± 1	14 ± 7
Mg 24 [a]	72 ± 10	157 ± 38	138 ± 42	150 ± 3	145 ± 7	285 ± 68	136 ± 25
Mg 26 [a]	82 ± 12	149 ± 26	136 ± 36	148 ± 3	141 ± 9	260 ± 58	136 ± 14
Al 27	225 ± 22	361 ± 62	437 ± 152	295 ± 6	322 ± 89	980 ± 185	509 ± 78
P31 [a]	98 ± 45	128 ± 106	126 ± 68	80 ± 2	100 ± 69	352 ± 98	196 ± 84
K 39 [a]	963 ± 93	572 ± 17	749 ± 142	561 ± 11	652 ± 98	596 ± 142	717 ± 202
Ca 43 [a]	75 ± 4	66 ± 3	78 ± 9	62 ± 1	64 ± 5	87 ± 16	65 ± 15
Ti 47	556 ± 31	215 ± 39	616 ± 23	251 ± 15	432 ± 20	166 ± 15	517 ± 30
V 51	-[a]	3 ± 1	2 ± 1	3 ± 0.05	2 ± 0.3	2 ± 1	2 ± 0.3
Cr 52	590 ± 47	533 ± 45	611 ± 67	644 ± 51	589 ± 64	683 ± 116	611 ± 55
Fe 54	1074 ± 55	2097 ± 322	2383 ± 1316	1508 ± 17	1397 ± 139	1756 ± 66	1605 ± 879
Mn 55	1740 ± 352	2003 ± 468	2166 ± 838	1490 ± 309	1480 ± 217	1693 ± 333	1601 ± 711
Fe57	1079 ± 55	2028 ± 465	2392 ± 1322	1528 ± 5	1407 ± 139	1765 ± 75	1596 ± 890
Co 59	0.8 ± 1[-b]	2 ± 1	2 ± 1	1.2 ± 0.05	2 ± 1	3 ± 1	4 ± 3.5
Ni60	6 ± 1	11 ± 3	12 ± 3	19 ± 0.1	20 ± 4	13 ± 8	26 ± 12
Cu63	87 ± 58	39 ± 10	54 ± 15	74 ± 42	68 ± 29	48 ± 22	126 ± 85
Zn 64	92 ± 46	243 ± 111	215 ± 128	70 ± 0	87 ± 25	610 ± 67	260 ± 107
Cu65	86 ± 57	40 ± 11	53 ± 17	81 ± 37	80 ± 35	65 ± 31	116 ± 78
Zn66	95 ± 46	248 ± 112	222 ± 132	75 ± 6	88 ± 26	622 ± 71	1243 ± 872

Csl (Cinsault), **Pn** (Pinot Noir), **CS** (Cabernet Sauvignon), **PB** (País base), **P** (País), **Mlb** (Malbec), **Cmn** (Carmenere). -[a] <LOD, [-b] < LOQ, [a] Concentration (mg L^{-1}).

Continuation

Elements	**Csl**	**Pn**	**CS**	**PB**	**P**	**Mlb**	**Cmn**
^{75}As	-^{a}	-^{a}	0.7 ± 0.1^{-b}	0.6 ± 0.1^{-b}	0.6 ± 0.2^{-b}	1.5 ± 2.3	0.7 ± 0.1^{-b}
^{77}Se	6 ± 1.3	5 ± 2	4.8 ± 1.4	2.2 ± 0.6^{-b}	3.7 ± 2^{-b}	4.3 ± 1.9^{-b}	4.9 ± 2.8
^{78}Se	6 ± 0.3	5 ± 0.3	5.0 ± 1.0	3.3 ± 0.4^{-b}	4.3 ± 1	4.1 ± 1.0^{-b}	4.8 ± 1.4
^{88}Sr	759 ± 95	1390 ± 54	1041 ± 431	200 ± 0	163 ± 59	361 ± 98	334 ± 295
^{98}Mo	2.1 ± 2^{-b}	1 ± 0.7^{-b}	0.9 ± 0.4^{-b}	3.6 ± 2.1	2.1 ± 2.0^{-b}	0.4 ± 0.1^{-a}	0.6 ± 0.3^{-a}
^{107}Ag	-^{a}	-^{a}	-^{a}	3.0 ± 1.6	-^{a}	-^{a}	-^{a}
^{111}Cd	-^{a}	-^{a}	-^{a}	0.2 ± 0.02	0.2 ± 0.1	0.2 ± 0.3	-^{a}
^{118}Sn	^{b}	$^{-a}$	$^{-a}$	1.4 ± 0.7	1.5 ± 0.5	$^{-a}$	$^{-a}$
^{121}Sb	1.0 ± 1.5	0.4 ± 4	$^{-b}$	0.4 ± 0.3	0.4 ± 0.3	$^{-b}$	$^{-b}$
^{138}Ba	506 ± 270	411 ± 222	416 ± 151	590 ± 20	509 ± 160	140 ± 35	321 ± 280
^{195}Pt	0.1 ± 0.1	0.04 ± 0.02	0.05 ± 0.04	0.60 ± 0.25	0.3 ± 0.2	0.04 ± 0.03	0.1 ± 0.1
^{202}Hg	-^{a}	-^{a}	-^{a}	1.06 ± 0.17	-^{a}	-^{a}	-^{a}
^{205}Tl	0.4 ± 0.05	0.38 ± 0.02	0.3 ± 0.1	0.25 ± 0.01	0.3 ± 0.3	0.4 ± 0.1	0.6 ± 0.3
^{208}Pb	2 ± 0,6	3 ± 2	2,7 ± 0,6	8 ± 0.1^{-b}	13 ± 10	1,4 ± 0,4	18 ± 14

Csl (Cinsault), **Pn** (Pinot Noir), **CS** (Cabernet Sauvignon), **PB** (País base), **P** (País), **Mlb** (Malbec), **Cmn** (Carmenere). -^{a} <LOD, $^{-b}$ < LOQ, ^{a} Concentration (mg L^{-1}).

STable 4- 8. The concentration of elements in grape according to different vineyard obtained with ICP - MS.

Red grape varieties within from the different vineyard						
	Neira			***Piedras del encanto***	***Puertas del Itata***	
Elements	**Csl**	**Pn**	**CS**	**Cmn**	**PB**	**P**
^{7}Li	6 ± 0,1	3 ± 0,1	3,6 ± 0,1	5 ± 0,2	5 ± 0,1	6 ± 0,7
^{9}Be	0,2 ± 0,02	0,3 ± 0,02	0,24 ± 0,02	0,2 ± 0,0	0,2 ± 0,02	0,1 ± 0,03
^{11}B	4097 ± 44	3005 ± 40	4762 ± 107	7276 ± 133	7786 ± 445	7672 ± 325
^{23a}Na	5 ± 0,4	3 ± 0,1	4 ± 0,1	7 ± 0,3	13 ± 0,5	15 ± 2
^{24a}Mg	64 ± 1,4	128 ± 4	135 ± 3	104 ± 2,0	150 ± 3	145 ± 7
^{26a}Mg	73 ± 1,7	130 ± 3	135 ± 3	118 ± 1,9	148 ± 3	141 ± 9
^{27}Al	242 ± 4,1	313 ± 8	360 ± 9	410 ± 8,2	295 ± 6	322 ± 89
^{31a}P	64 ± 1,3	46 ± 1	131 ± 4	105 ± 1,5	80 ± 2	100 ± 69
^{39a}K	1034 ± 19	581 ± 15	726 ± 8	977 ± 14	561 ± 11	652 ± 98
^{43a}Ca	72 ± 1,3	66 ± 3	83 ± 3	83 ± 2	62 ± 1	64 ± 5
^{47}Ti	322 ± 15	255 ± 11	761 ± 30	673 ± 36	253 ± 10	429 ± 202
^{51}V	1 ± 0,1	4 ± 0,2	3 ± 0,05	1 ± 0,01	3 ± 0,05	2 ± 0,3
^{52}Cr	558 ± 7.5	497 ± 10	568 ± 8	598 ± 15	64 ± 5	597 ± 62
^{54}Fe	1105 ± 26	2343 ± 46	1403 ± 19	1888 ± 25	1508 ± 17	1397 ± 139
^{55}Mn	1480 ± 48	1692 ± 308	2198 ± 654	2205 ± 117	1490 ± 309	1480 ± 217
^{57}Fe	1112 ± 23	2350 ± 42	1407 ± 12	1863 ± 21	1528 ± 5	1407 ± 139
^{59}Co	0,4 ± 0,2	1 ± 0,4	2 ± 0,1	7 ± 5	1 ± 0,05	1,5 ± 0,8
^{60}Ni	5 ± 0,2	13 ± 1	9 ± 0,2	14 ± 2	19 ± 0,1	20 ± 3,9
^{63}Cu	131 ± 1,2	46 ± 7	63 ± 1	40 ± 3	74 ± 42	68 ± 29
^{64}Zn	57 ± 8,2	158 ± 20	150 ± 21	385 ± 10	70 ± 0	87 ± 25
^{65}Cu	130 ± 0	47 ± 8	62 ± 4	40 ± 0	81 ± 37	80 ± 35

[a] (mg L^{-1}).

Continuation

Red grape varieties within from the different vineyard

	Neira			***Piedras del encanto***	***Puertas del Itata***	
Elements	**Csl**	**Pn**	**CS**	**Cmn**	**PB**	**P**
^{66}Zn	60 ± 11	162 ± 23	155 ± 23	390 ± 8,2	75 ± 6	88 ± 26
^{75}As	0,5 ± 0,04	0,4 ± 0,03	0,7 ± 0,1	0,5 ± 0,0	1 ± 0,1	0,6 ± 0,2
^{77}Se	5 ± 1	3,9 ± 2	5 ± 0,5	8 ± 2,7	2 ± 1	3,7 ± 1,7
^{78}Se	6 ± 0,4	5,3 ± 0,2	6 ± 0,3	6 ± 0,4	3 ± 0,4	4,3 ± 1,0
^{88}Sr	688 ± 11,7	1357 ± 27	1362 ± 33	113 ± 2,5	200 ± 0	163 ± 59
^{98}Mo	3 ± 2,7	0,8 ± 0,1	1,3 ± 0,2	0,4 ± 0,02	4 ± 2,1	2,1 ± 2,0
^{107}Ag	3 ± 1,1	1,4 ± 0,1	1,4 ± 0,1	1,1 ± 0,1	3 ± 1,6	2,0 ± 1,5
^{111}Cd	0,08 ± 0,01	0,1 ± 0,01	0,2 ± 0,02	0,1 ± 0,02	0,2 ± 0,02	0,2 ± 0,1
^{118}Sn	1 ± 0.6	0.4 ± 0,1	0.2 ± 0,1	0.1 ± 0,01	1 ± 0.7	2 ± 0.5
^{121}Sb	0.2 ± 0.1	0.2 ± 0,04	0.2 ± 0,1	0.1 ± 0,02	0.4 ± 0.3	0.4 ± 0.3
^{138}Ba	715 ± 21	583 ± 8	582 ± 19	680 ± 20	590 ± 20	509 ± 160
^{195}Pt	0,2 ± 0,14	0,05 ± 0,01	0,03 ± 0,01	0,02 ± 0,01	0,6 ± 0,3	0,3 ± 0,2
^{202}Hg	0,7 ± 0,11	0,6 ± 0,03	0,5 ± 0,1	0,4 ± 0,03	1,1 ± 0,2	0,7 ± 0,3
^{205}Tl	0,4 ± 0,01	0,4 ± 0,01	0,3 ± 0,0	0,2 ± 0,01	0,3 ± 0,01	0,3 ± 0,3
^{208}Pb	2 ± 0,7	4 ± 1	3 ± 0,2	2 ± 0.3	8 ± 0,1	13 ± 10

^{a} (mg L^{-1}).

Continuation

Red grape varieties within from the different vineyard

	Entre Valles		*Chillan*			
Elements	**CS**	**Csl**	**Cmn**	**Csl**	**CS**	**Mlb**
^{7}Li	11 ± 0.1	8 ± 2	1 ± 0,1	14 ± 0,9	7,5 ± 1,3	1 ± 0.2
^{9}Be	0.6 ± 0.02	0.3 ± 0.03	0,1 ± 0,1	0,2 ± 0,04	0,4 ± 0,1	0.2 ± 0.3
^{11}B	8463 ± 101	3568 ± 70	8949 ± 540	3188 ± 216	10937 ± 2264	6132 ± 4959
^{23a}Na	61 ± 0.9	11 ± 0.1	17 ± 7	15 ± 0,6	6 ± 1	5 ± 0.4
^{24a}Mg	90 ± 1	82 ± 1	152 ± 8	83 ± 4,8	164 ± 47	224 ± 61
^{26a}Mg	103 ± 1	94 ± 1	145 ± 8	95 ± 5	153 ± 47	208 ± 61
^{27}Al	265 ± 6	411 ± 21	562 ± 36	200 ± 8	580 ± 104	713 ± 352
^{31a} P	133 ± 2	113 ± 2	229 ± 73	149 ± 9	119 ± 106	288 ± 101
^{39a} K	844 ± 9	1216 ± 11	582 ± 83	856 ± 6	718 ± 206	529 ± 86
^{43a} Ca	65 ± 2	67 ± 1	58 ± 10	80 ± 2	80 ± 7	85 ± 19
^{47}Ti	820 ± 29	712 ± 28	433 ± 345	918 ± 61	386 ± 165	170 ± 74
^{51}V	0.9 ± 0.06	1 ± 0.2	2 ± 0,2	0,9 ± 0,1	2 ± 0,1	3 ± 1
^{52}Cr	580 ± 8	529 ± 26	616 ± 68	633 ± 30	661 ± 81	664 ± 109
^{54}Fe	1225 ± 33	2835 ± 52	1686 ± 1213	1028 ± 56	3696 ± 806	1760 ± 65
^{55}Mn	961 ± 24	3508 ± 62	1440 ± 782	2131 ± 169	2745 ± 429	2003 ± 581
^{57}Fe	1233 ± 38	2844 ± 51	1685 ± 1235	1030 ± 55	3711 ± 808	1677 ± 278
^{59}Co	2 ± 0.1	3 ± 1	2 ± 1	1,4 ± 0,8	3,3 ± 1	3 ± 1
^{60}Ni	10 ± 0.4	10 ± 2	32 ± 11	7 ± 0,2	15 ± 3	11 ± 8
^{63}Cu	33 ± 1	48 ± 1	171 ± 81	20 ± 1	57 ± 14	42 ± 23
^{64}Zn	80 ± 8	140 ± 5	202 ± 69	145 ± 6	332 ± 99	503 ± 152
^{65}Cu	30 ± 0	48 ± 1	154 ± 68	20 ± 0,0	57 ± 18	57 ± 35
^{66}Zn	83 ± 5	142 ± 4	1617 ± 805	148 ± 5	341 ± 104	514 ± 159

^{a} (mg L $^{-1}$).

Continuation

Red grape varieties within from the different vineyard

	Entre Valles		*Chillan*			
Elements	**CS**	**Csl**	**Cmn**	**Csl**	**CS**	**Mlb**
^{75}As	0.7 ± 0.02	1 ± 0	1 ± 0,1	0,4 ± 0,1	0,6 ± 0,0	1 ± 2
^{77}Se	6 ± 2	8 ± 2	3 ± 2	6,1 ± 2	4,4 ± 1,6	4 ± 2
^{78}Se	5 ± 0.04	6 ± 0.5	4 ± 1	5,8 ± 0,3	4,5 ± 1,3	4 ± 1
^{88}Sr	1113 ± 17	897 ± 19	448 ± 297	865 ± 44	764 ± 520	942 ± 535
^{98}Mo	1 ± 0.04	1 ± 0.1	0,7 ± 0,3	1 ± 0,3	0,5 ± 0,1	1 ± 1
^{107}Ag	1 ± 0.05	1 ± 1	1,4 ± 0,5	2 ± 1	1,1 ± 0,2	2 ± 1
^{111}Cd	0.1 ± 0.01	0.1 ± 0.01	0,5 ± 0,2	0,1 ± 0,03	0,1 ± 0,01	0.2 ± 0.3
^{118}Sn	0.2 ± 0.01	0.2 ± 0.04	0.4 ± 0.2	0.1 ± 0.1	0,2 ± 0.1	0.3 ± 0.1
^{121}Sb	0.2 ± 0.01	0.2 ± 0.1	2 ± 1	0,2 ± 0,2	0.1 ± 0,03	0.3 ± 0.5
^{138}Ba	450 ± 48	367 ± 32	164 ± 125	193 ± 10	274 ± 83	155 ± 29
^{195}Pt	0.02 ± 0.005	0.1 ± 0.1	0,1 ± 0,1	0,02 ± 0,01	0,1 ± 0,0	0.1 ± 0.03
^{202}Hg	0.5 ± 0.06	1 ± 0.2	0,5 ± 0,1	0,4 ± 0,1	0,5 ± 0,1	0.5 ± 0.1
^{205}Tl	0.1 ± 0	0.2 ± 0.003	0,8 ± 0,2	0,3 ± 0,0	0,4 ± 0,1	0.4 ± 0.1
^{208}Pb	2.9 ± 0.9	3 ± 2	24 ± 11	2 ± 0.3	2.3 ± 0.5	1 ± 0.4

[a] (mg L^{-1}).

STable 4- 9. Descriptors for grapes of each vineyard according to a geographical area.

Vineyard	Varieties	Descriptors
Neira	*Csl*	Pt, Sb, Ag, Tl, Se, Li, Ba, Cu, K, Na, Cr, B, Ca, P and Ti.
	CS	Al, Co, Mn, Mg, Sr, and Zn.
	Pnt	Fe, Cr, Be, Ni and Pb.
Piedras	*M*	Zn, Pb, Ag, Sb, Na, Pt, Tl, Cu, Sr.
	Csl	Mg, be Li, Cu65, Fe, Mg, Mn, Al, B, Ni.
	Cmn	Ba, P, Ca, Se, Ti, Co, Cr.
Puertas	*Ps*	K, Se, Ti, Na.
	Pb	Ag, Sb, Be, Pt.
	Ch	Sr, Ca, Fe, Ni, Pb, Zn, Co.
Entre V	*Csl*	K, Mn, Cu, Fe, Zn, Tl, Al, Co, Se, Pt, Ca, Se, Sb, Ag, Ni.
	CS	Sr, B, Na, P, Mg, Be, Cr, Ti, Cr, Ba, Ti, Pb.
Chillan	*Cmn*	Pb, Tl, Ni, Cu, Zn.
	Sb	Na, B, Ti.
	mlb/CS	Zn, Fe, Cr, Mg, Co, Al, Ca, Be, Ba.
	csl	Li, Se, Sr, Ag, Mn, Sb.

STable 4- 10. Calibration developed of SEC column with PEO/PEG.

MW Da	**t_R (time)**	**Log (MW)**
232	23.57	2.4
599	22.12	2.8
1960	21.88	3.3
6690	9.59	3.8
18600	9.30	4.3

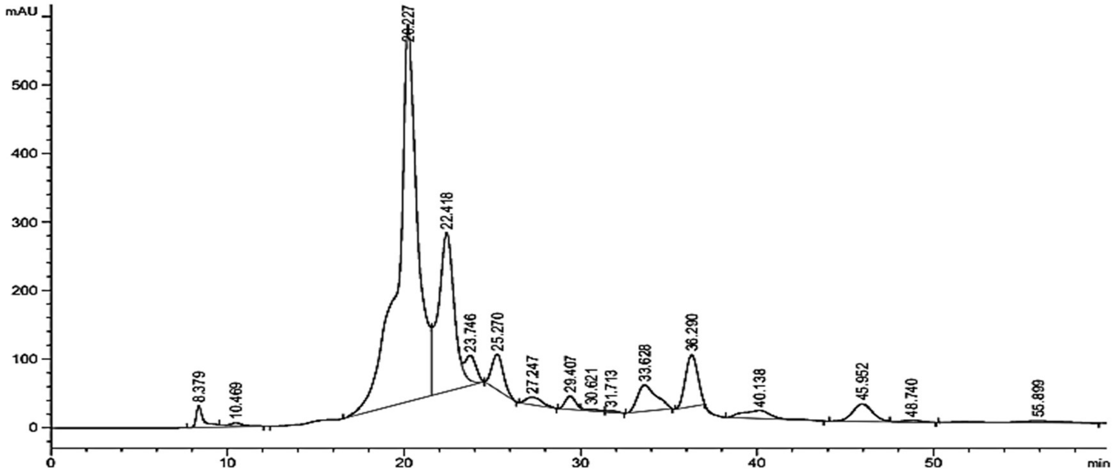
STable 4- 11. Samples and retention time of SEC fractions in red and white wine.

Red wine fractions

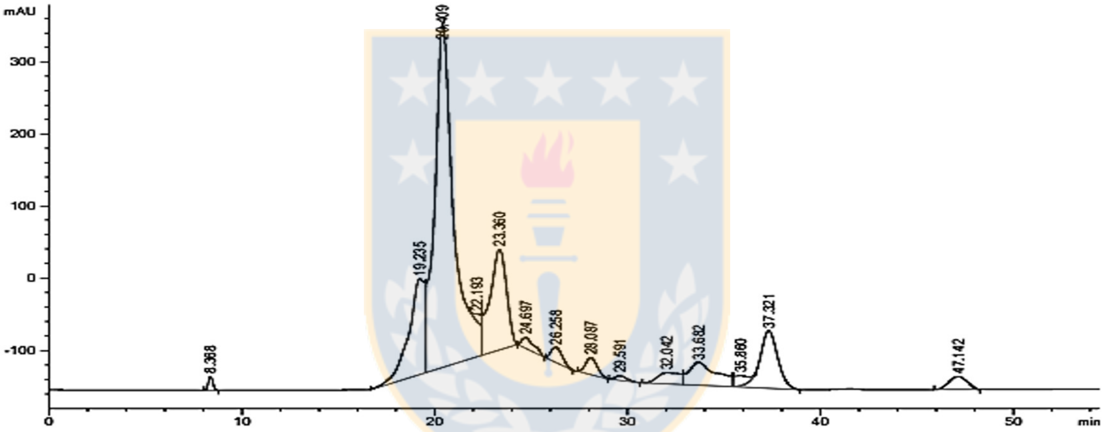
	tr min				
code	Cabernet Sauvignon	Pinot noir	Cinsault	Carmenere	Malbec
F1	17 - 19	16.2 - 17.4	17.3 - 18.7	16.7 - 19.2	17.9 - 21.4
F2	20 - 21.3	17.5 - 18.8	18.8 - 19		22.5 - 24.3
F3	21.4 - 22.9	18.9 - 19.9	20 - 21.1		24.4 - 26.3
F4	23 - 24.4	20 - 21.5	22.9 - 24.3		
F5	24.5 - 25.5	21.8 - 22.9	24.8 - 26.1		
F6		23 - 24			
F7		24.7 - 26.1			

White wine fractions

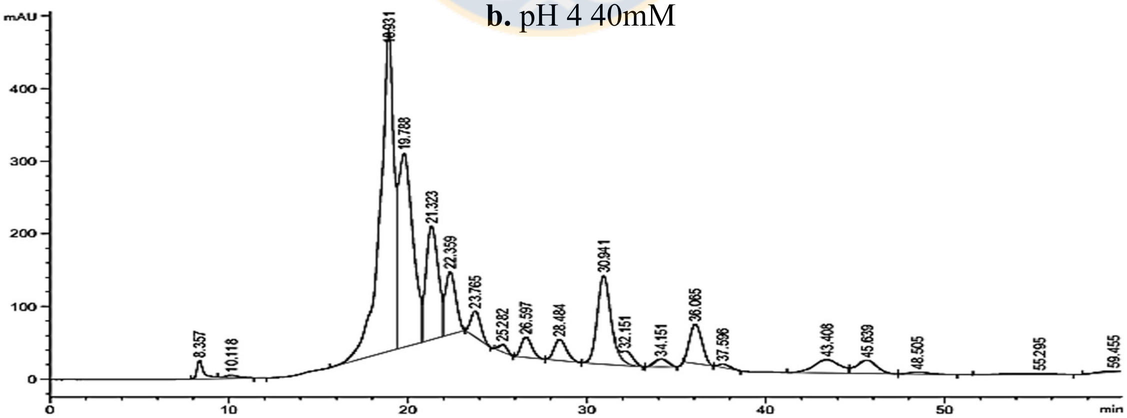
	tr min		
code	Chardonnay	Muscat of A	Sauvignon Blanc
F1	17 -19	10 - 11	17 - 21
F2	20 - 21.3	16 - 21	22.8 - 24.5
F3	21.4 - 22.9	24 - 26	24.5 - 26.5



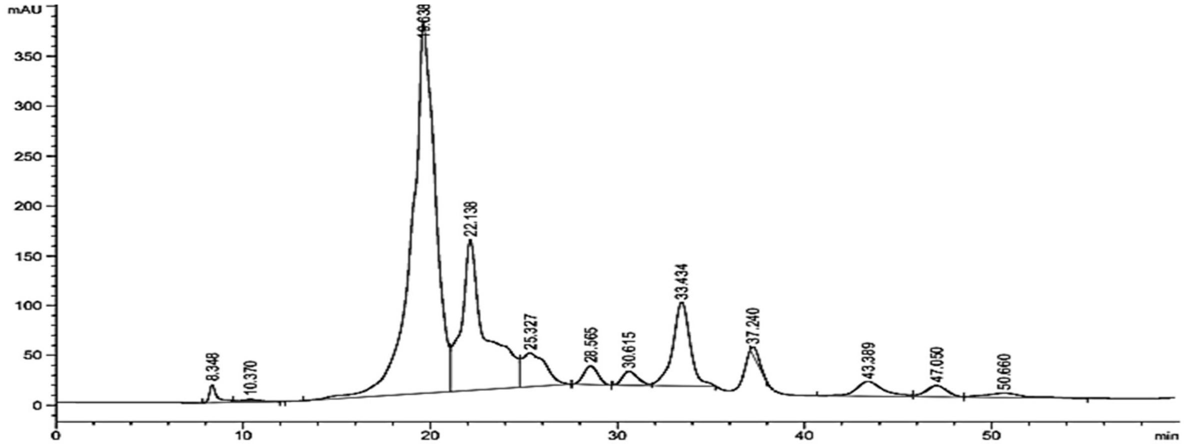
a. pH 4 10mM



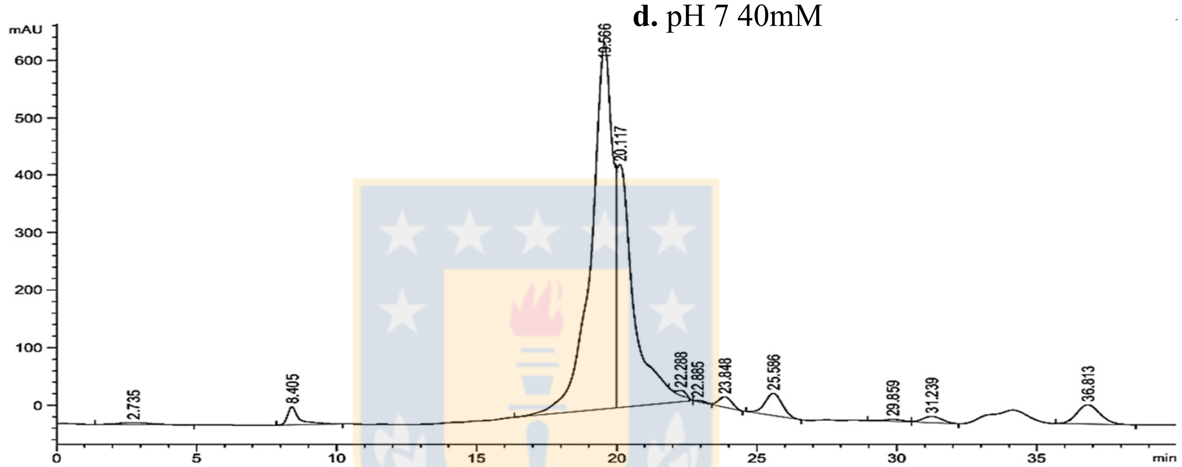
b. pH 4 40mM



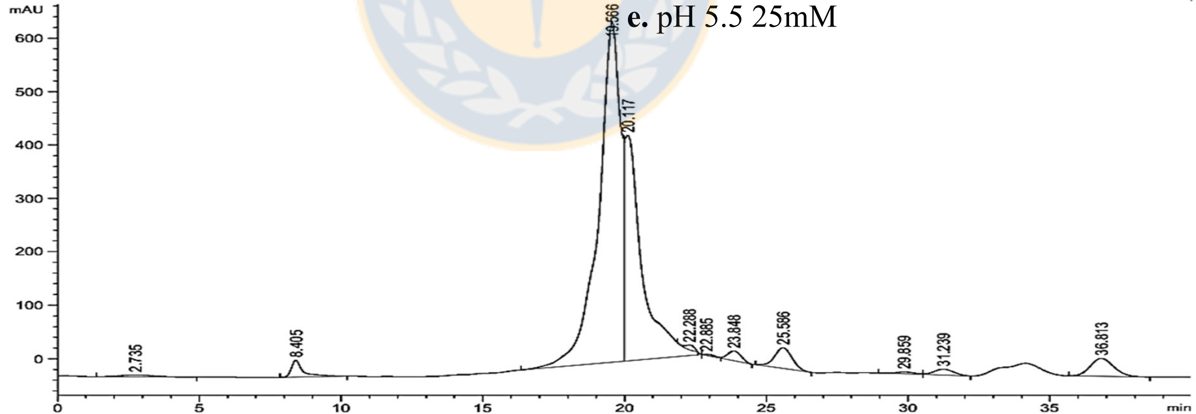
c. pH 7 10mM



d. pH 7 40mM

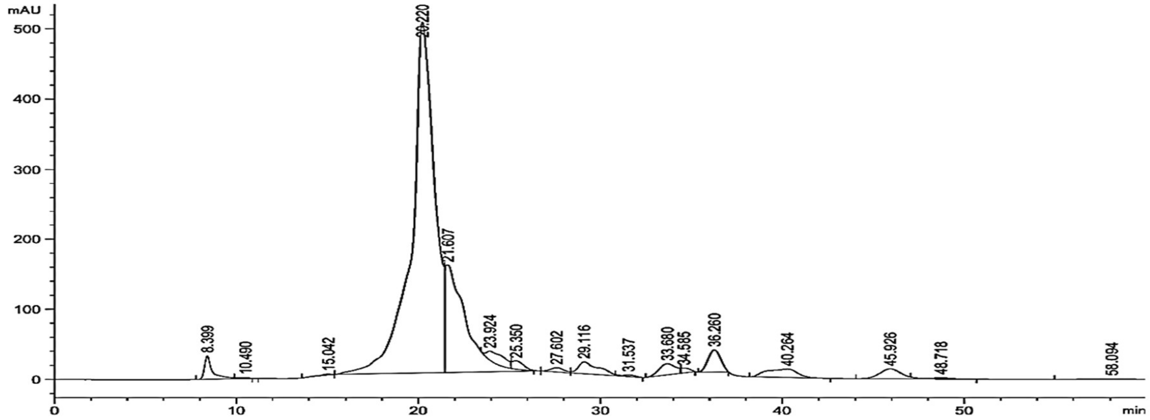


e. pH 5.5 25mM

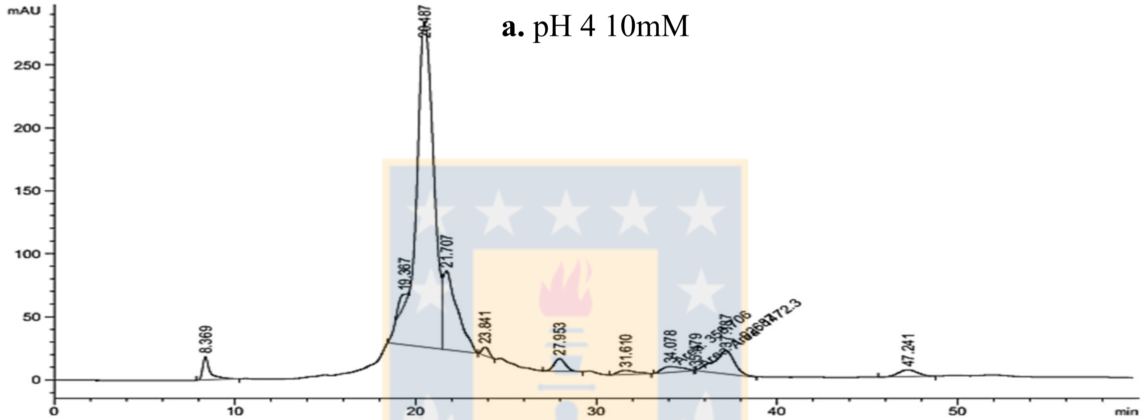


f. pH 6.5 25mM

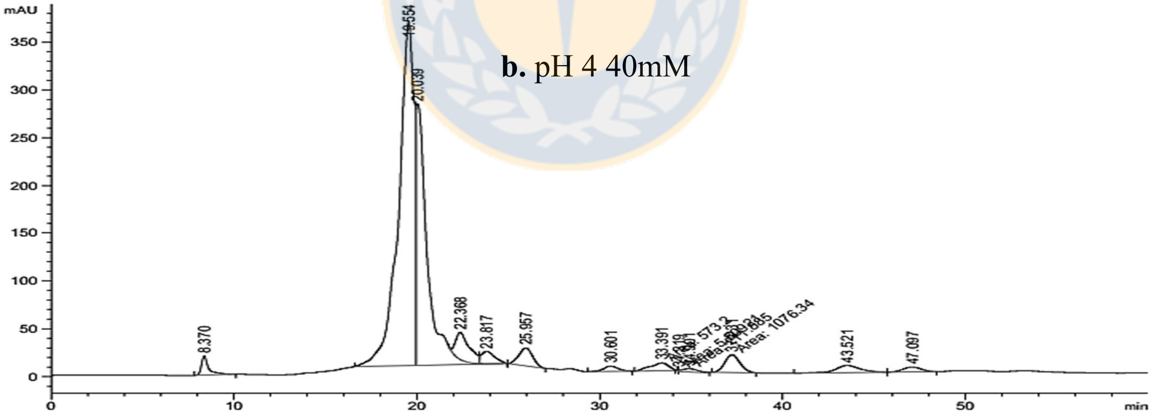
SFigure 4- 1. Optimization of mobile phase and pH of red wine.



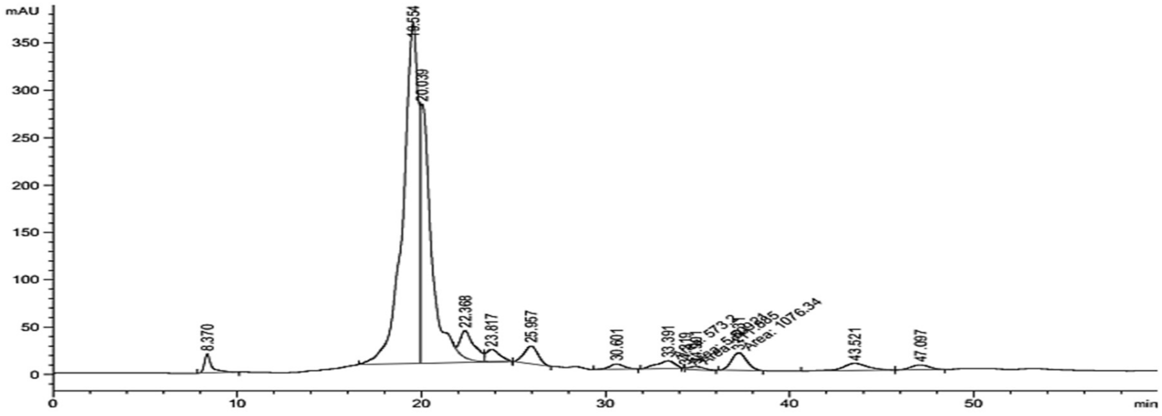
a. pH 4 10mM



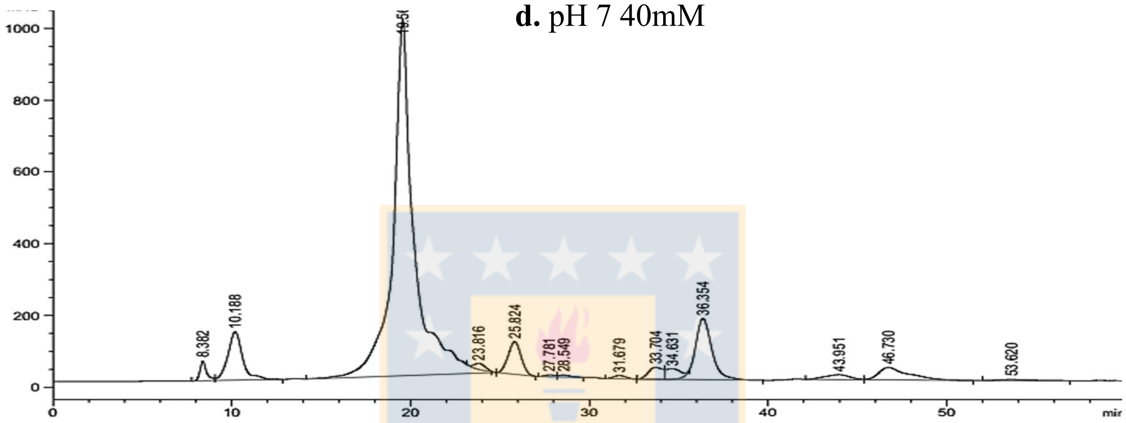
b. pH 4 40mM



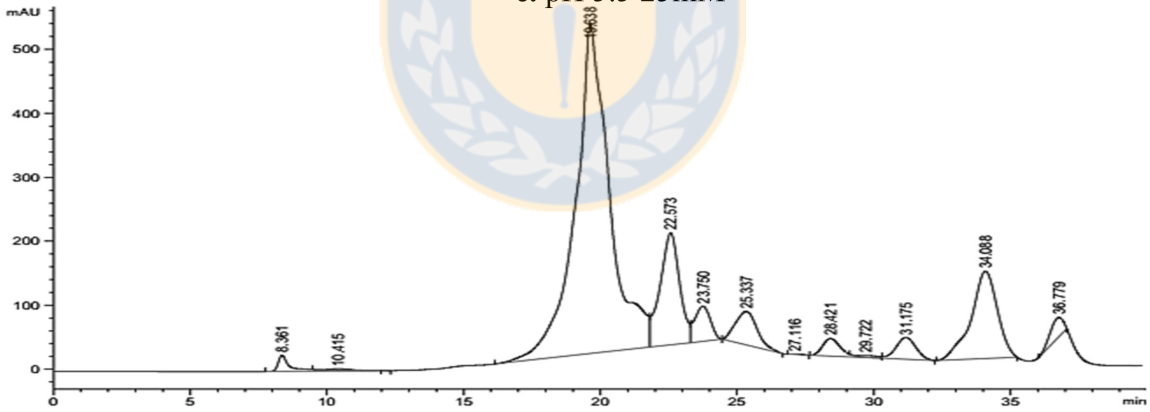
c. pH 7 10mM



d. pH 7 40mM

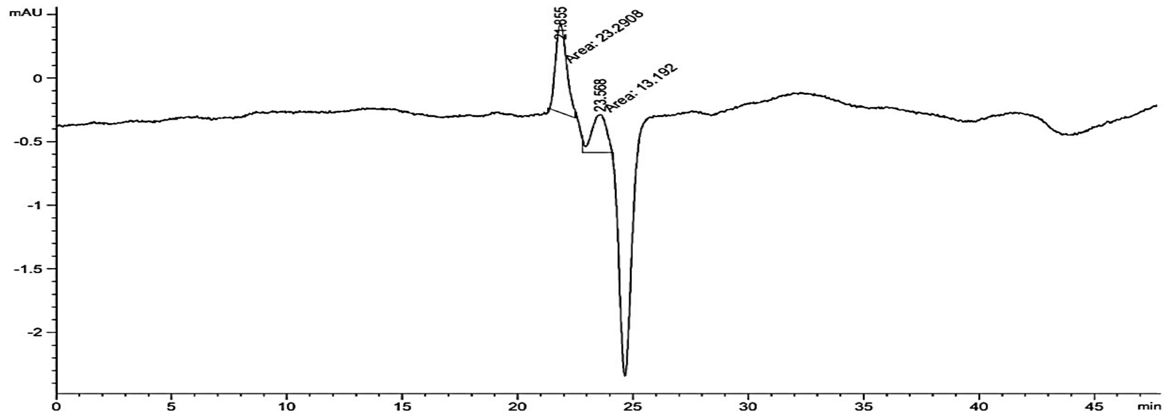


e. pH 5.5 25mM

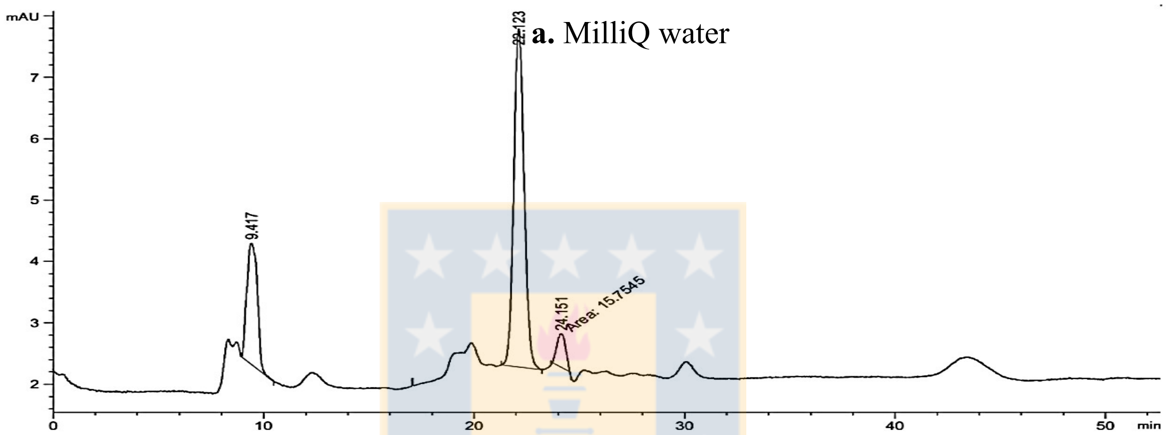


f. pH 6.5 25mM

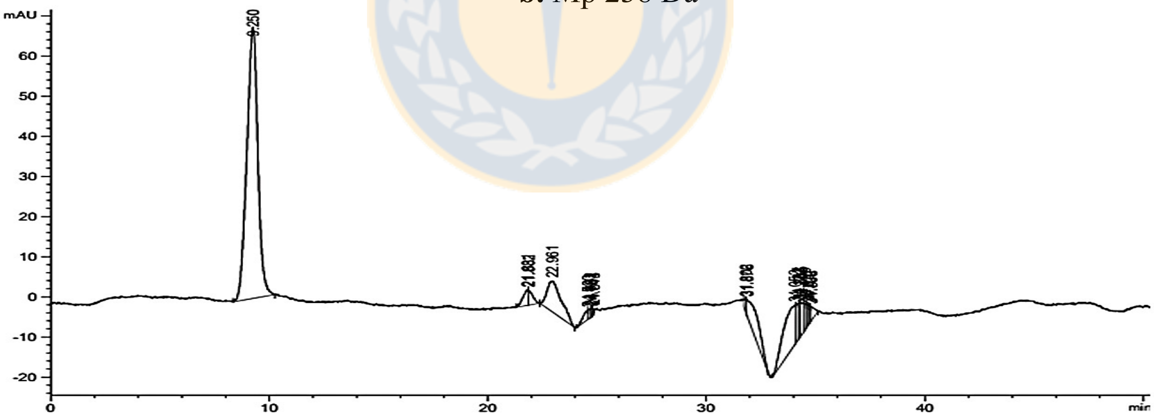
SFigure 4- 2. Optimization of mobile phase and pH of white wine.



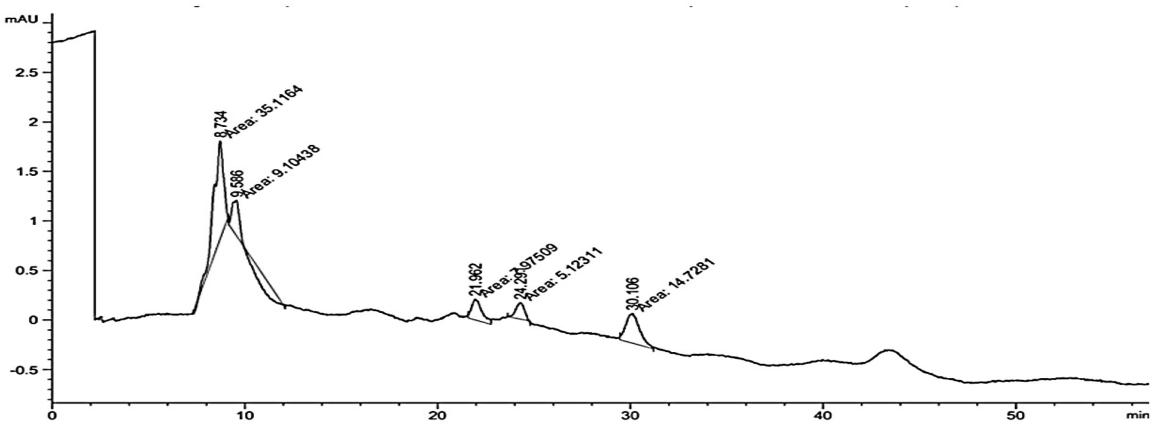
a. MilliQ water



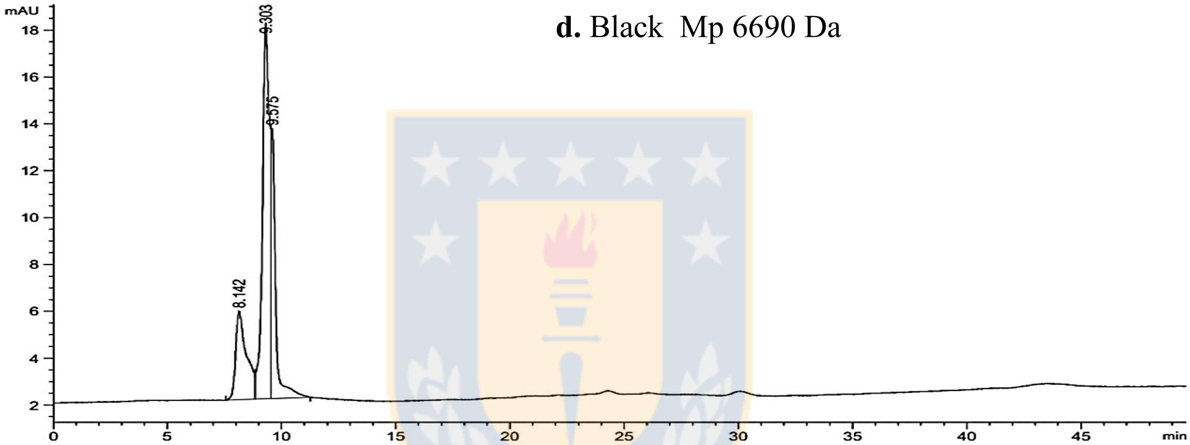
b. Mp 238 Da



c. Mp 599 Da

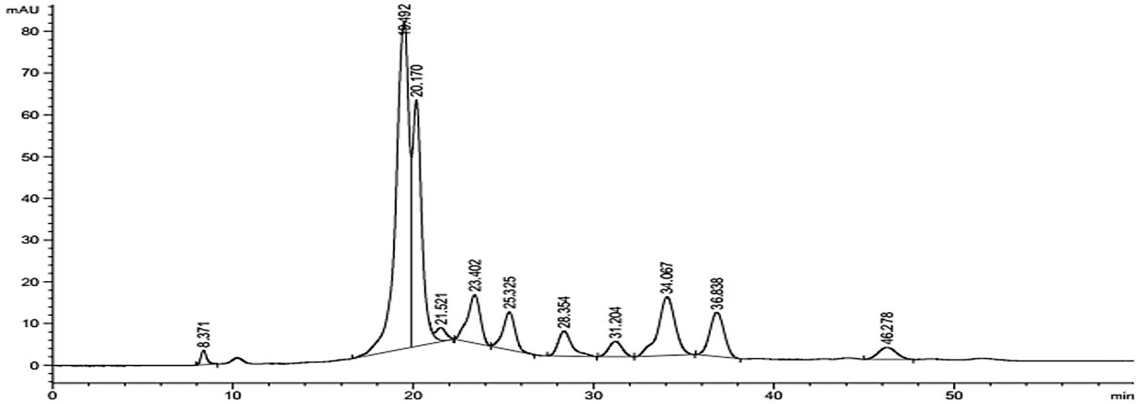


d. Black Mp 6690 Da

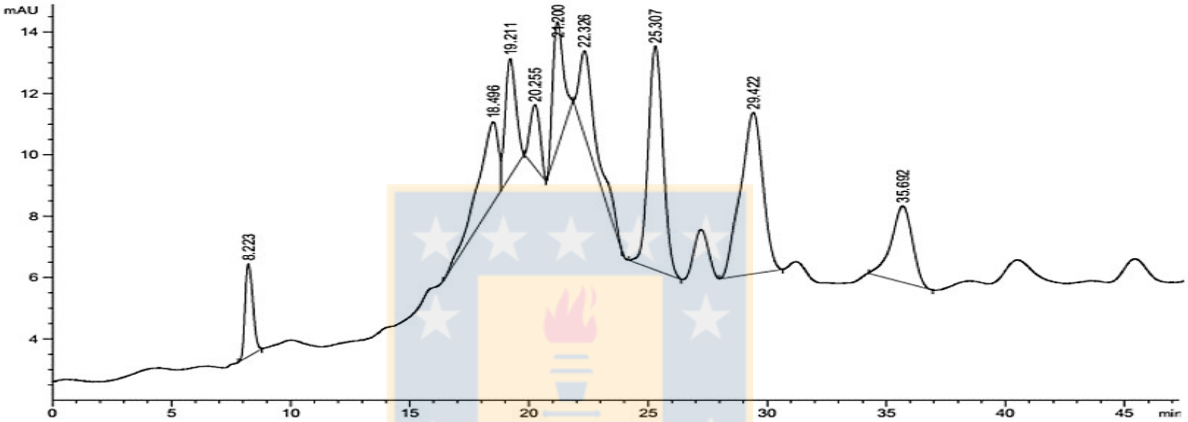


e. Dextran

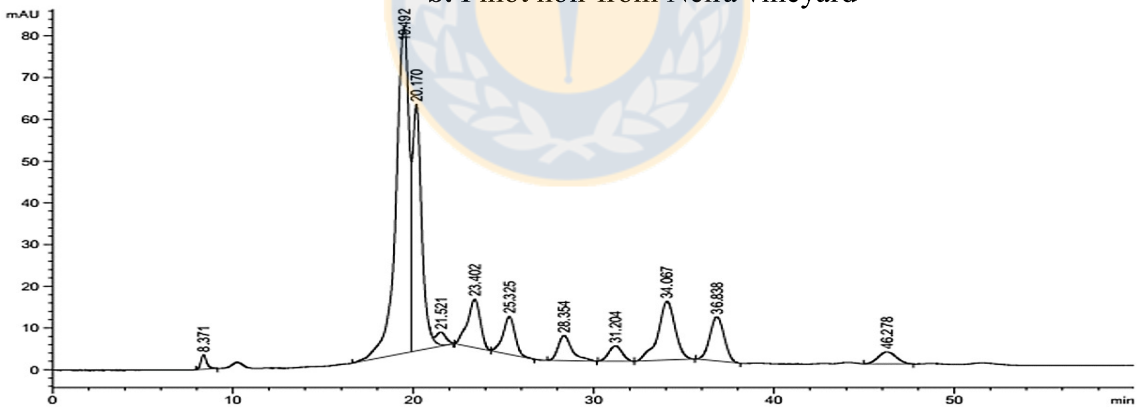
SFigure 4- 3. Column calibration was performed with calibration PEG/PEO Kits (molecular weights range 238 – 6690 Da). The exclusion volume (V_0) was determined using Blue dextran, while the permeation volume (Vp) was fixed by injecting Milli-Q water



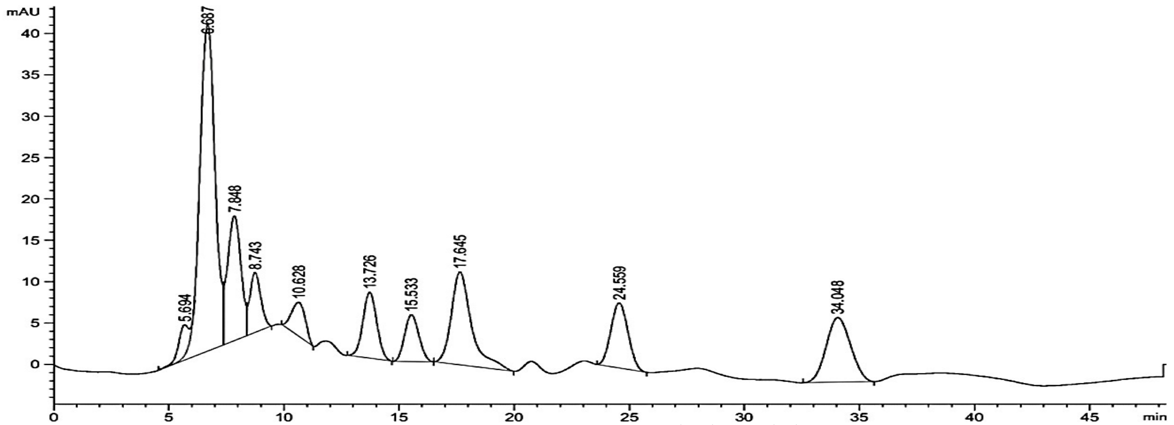
a. Cabernet Sauvignon from Neira vineyard



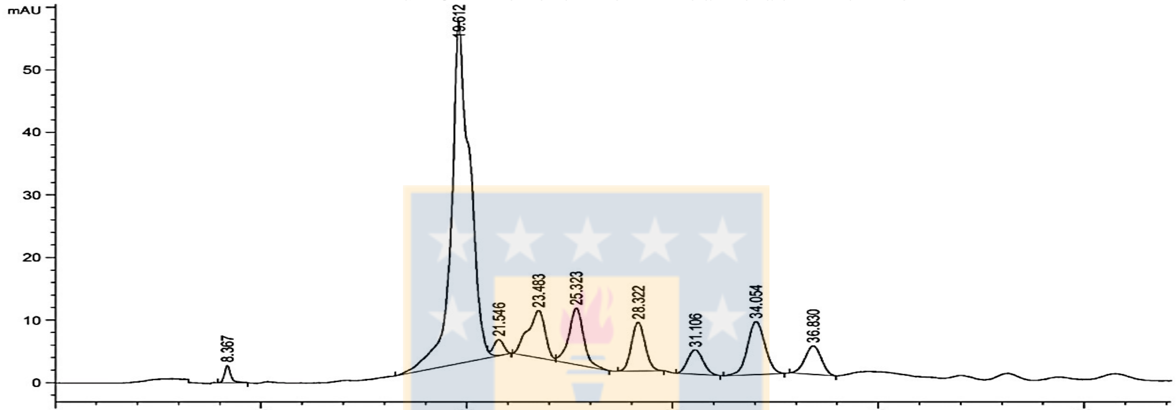
b. Pinot noir from Neira vineyard



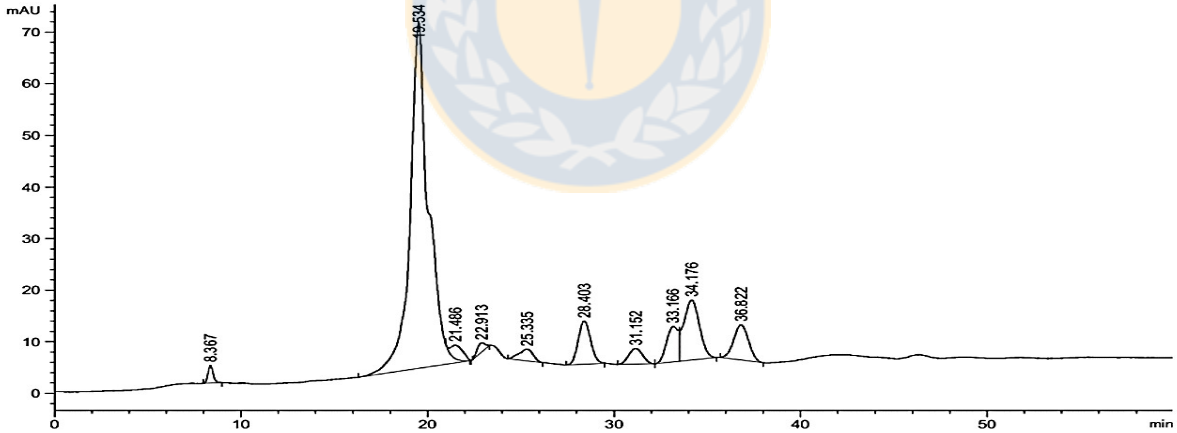
c. Cinsault from Entre Valles vineyard



d. Carmenere From Piedras del Encanto



e. Carmenere From Chillan



f. Malbec From Chillan

SFigure 4- 4. The chromatograms profiles for each wine from the SEC analysis.

5. Bioavailability of trace and ultra trace metals in Chilean wines from Itata valley

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Manuscrito en revisión para ser enviado a Food Chemistry

Abstract

Itata Valley, located in the South Valley, comprises an area bordered by the Andes and a lower coastal mountain, and it is one of the most important winemaking emerging areas in Chile. Current trends regarding winery practices imply the production of wines made without chemical and minimum technological intervention in growing grapes, and in winery practices. Chemical characterization of these wines is scarce, and data regarding bioavailability of essential, and toxic compounds, are not available.

The aim of the current work has been the assessment of the bioavailable fraction of trace and ultra trace metals in Chilean 'natural wines'. An *in vitro* bioavailability approach based dialyzability has been applied. The *in vitro* digestion procedure consisted of two sequential stages, which simulate gastric and intestinal digestion. The first step requires 0.15 g of a freshly prepared gastric solution [6.0 % (m/v) pepsin in 6.0 M hydrochloric acid, and incubation at 37 ºC with an orbital – horizontal shaking at 150 rpm for 120 min. The second step uses 5.0 ml of intestinal solution (4.0 %(m/v) pancreatin and 2.5 %(m/v)) bile salts dissolved in 0.1 M sodium hydrogen carbonate, and also incubation at 37 ºC with an orbital – horizontal shaking at 150 rpm for 120 min]. During this second stage, dialysis membranes (10 kDa cut-off) filled with 20 mL of 0.15 N PIPES (pH 7.5) were used for simulating bio-absorption. Total concentrations of trace and ultra trace metals in wines were directly assessed by inductively coupled plasma – mass spectrometry (ICP-MS) after 1:10 dilution with 1.0 % (v v^{-1}) nitric acid. Similarly, dialysates analysis has also been performed by ICP-MS after 1:5 dilution in ultrapure water. Bio-availability ratios (dialyzability) were finally obtained by calculating the percentages of total

5.1. Introduction

The consumption of wine in the human diet is considered a promising source for a healthy life, mainly because of the high nutritional value regarding essential minerals, trace elements and bioactive compounds [1–5]. The contents of trace metals in wine depend on endogenous and exogenous factors including the type of vineyard soil, the metal intake from the soil into the grapes, grape variety and maturity, climatic conditions, viticulture management practices, environmental pollution, winemaking additives, winery equipment, wine preservation, and bottling [6,7].

However, a fingerprint to each wine by the elemental content a low differentiation of wines from one region to another [8–10]. Elements are extensively divided into major and trace elements. Major elements include, calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), chloride (Cl), phosphorus (P) and sulfur (S); whereas, , trace elements are iodine (I), zinc (Zn), selenium (Se), iron (Fe), manganese (Mn), copper (Cu), cobalt (Co), molybdenum (Mo), fluoride (F), chromium (Cr) and boron (B) [11].

Several approaches have been developed to assess the bioaccessibility of essential trace elements from different food matrices by mimicking the human digestive process; most of them use in vitro experiments [11]. *(e.g.,* seaweeds, artichoke heads, plant foods, among others) [12–14].

Metals have key roles in our body to do necessary functions [15], but they can also be dangerous and tends to be accumulated in the organisms. The bioavailability of metals may vary depending on their release from the food matrix, their stability against some biochemical factors, and their absorption capacities [4].

No data reported in the literature was found regarding the bioavailability of the mineral profile of wine of Itata valley from Chile. In our knowledge, this is the first study specifically focused on the evaluation of the bioavailability trace elements content of wine using a simulated in vitro digestion system, and the analytical determinations were made by ICP-MS.

In this work, a source of information regarding the levels of total and bioavailable trace and ultra trace elements in wine samples collected at different geographic points of Itata valley from Chile is given.

5.2. Materials and methods

5.2.1. Instrumentation

Metal content was measured with a Perkin Elmer Nex-Ion 300X ICP-MS instrument (Waltham, MA, USA) equipped with a steadfast SC2 DX autosampler (Elemental Scientific, Omaha, NB, USA). The argon gas with a minimum purity of 99.996% was supplied by Air Liquide. An HF- resistant and high-performance perfluoracetate (PFA) nebulizer PFA-ST model, coupled to a quartz-made baffled cyclonic spray chamber, cooled with The PC 3 system from ESI was used. Box cut incubator situated on a Rotabit orbital-rocking platform shaker (J.P. Selecta, Barcelona, Spain) was used for temperature control of the enzymolysis procedure.

Dialyzability was performed using Cellu Sep® H1 high grade regenerated cellulose tubular membranes (molecular weight cut-off 10 kDa, 50 cm length, 25.5 mm diameter dried and a volume to length ratio of 5.10 ml cm-1) obtained from Membrane Filtration Products Inc. Seguin, TX, USA).. The chemometrics package used for data analysis was STATGRAPHICS Plus 5.1 (Manugistics Inc., Rockville M.D., USA).

5.2.2. Reagents

Deionized water 18.2 MΩ cm, was obtained from a Milli-Q Gradient A1, USA. Standard solutions used for the calibration curves were prepared by dilution of stock analytical standards (1000 mg L^{-1}) supplied by Merck (Poole, Dorset, UK). Digestive enzymes (porcine pepsin, p-7000, porcine pancreatin, P-1750), bile salts (approx. 50% sodium cholate and 50% sodium deoxycholate) and piperazine-NN-bis(2-ethane-sulfonic acid) di-sodium salt (PIPES), were obtained from Sigma Chemicals (St. Louis, MO, USA). Sodium hydrogen carbonate was from Merck. Nitric acid 69%, hydrochloric acid 37% and hydrogen peroxide 33% (m/v) were from Panreac (Barcelona, Spain).

5.2.3. Wines samples

A collection of eighteen varietal and young wine samples were obtained directly from the different wine producers and collected of the fermentation tanks before the final process of winemaking and after of alcohol fermentation.

The wine samples were collected from the southern wine region in Chile at subregion denominated Itata valley according to Decree No. 464 local wine legislation. For this study, the varieties Cinsault, Muscat of Alexandria, Cabernet Sauvignon, Carmenere, Malbec and Pinot noir were investigated. The set includes 14 red wines and 3 white wines. Wine samples were collected in polypropylene bottles and stored at 5 °C. Each sample was analyzed in duplicate and analyzed in triplicate.

5.2.4 Metal Bioavailability procedure

The in-vitro dialyzability procedure is adapted from the protocol by [12]. Samples were subjected in triplicate to an in vitro dialyzability. Wine (5mL) was diluted with 15 mL of ultrapure water in a wide-mouth Erlenmeyer flask, and after five minutes for homogenization, the pH was adjusted to 2.0 HCl 0.1M.

The gastric solution (0.15 g pepsin) was added and then, the mixture was thermostated at 37 ° C under orbital – horizontal shaking (37 °C, 170 rpm) for 120 min. Gastric digestion was halted by immersing the flasks in an ice-bath. The procedure continued by adding 5 ml of 4.0% (m/v) pancreatin and 2.5% (m/v) bile salts solution prepared in 0.1 M sodium hydrocarbonate, for the intestinal digestion stage with dialysis.

Dialysis membranes (10 kDa MWCO), filled with 20 ml of a 0.15N PIPES solution (pH 7.5) were placed inside the flasks. Intestinal digestion took place under orbital – horizontal shaking (37 °C, 170 rpm) for 120 min, after which it was halted by immersing the flasks into an ice-bath. Dialyzates (solution contained in the dialysis membrane) and the residual or no dialyzable fraction (slurries remaining in the flask) were transferred to polyethylene vials and weighed separately.

The acceptor solution (PIPES 0.15 N) constitutes the dialysate, and the amount of metals in it represents the bioavailable fraction (a fraction that is capable of being digested and which is capable of being assimilated by the intestine). The bioavailable fraction was calculated as the quotient between the concentration in the dialysate and the total concentration multiplied by one hundred.

5.2.5. ICP-MS measurements

Metals in wines (after 1:10 dilution with 1% (v/v) HNO_3) and in the dialyzates were also measured by ICP-MS under standard operating conditions at 0.8 ml min^{-1} by applying 1000 W RF power and 0.85 l min^{-1} nebulizer (operating parameters are described in Stable5- 1).

Metals measured were: Li, Be, B, Na, Mg, Al, K, Ca, Ti, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Pt, Hg, Tl, and Pb. All samples were filtered through 0.20 µm cellulose acetate syringe filters (Millipore) before ICP-MS measurements. Determinations were performed by using the standard addition method. For potassium and calcium, a dilution of 1:100 was necessary. Volumetric equipment was soaked in 1% distilled HNO_3 overnight and thoroughly rinsed with Milli-Q water, before use.

High-purity ethanol was used for preparing matrix-matched standards. The calibration range for K, Ca, Mg, Al, Fe, Na, and Sr were through 0 to 10 mg L$^{-1.}$ Calibrations for trace elements Li, Be, B, V, Cr, Ti, Se, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sn, Sb, Ba, Pt, Tl, and Pb were up to 200 µg L^{-1}, and for Hg up to 10 µg L^{-1}. Blank solutions were prepared with the same ethanol volume than those found in wines under study.

Tellurium, yttrium, indium, germanium, rhodium, and scandium were selected as internal standards at 10 µg L^{-1}. The LODs and LOQs (3/10 SD criterion, S.D. standard deviation of eleven measurements of a reagent blank) expressed as µg L^{-1} for wine are listed in Stable 5- 2.

Regarding metals assessment in dialyzates, the calibrations were based on 0.15N PIPES standard aqueous solutions covering metal concentrations from 0 to 50 µg L^{-1} for all elements, except for Cu and Zn (0–100 µg L^{-1} range), and Al, Ca, Fe, K, Mg, and P (0 to

2500 μg L^{-1} range). Yttrium and indium (5 μg L^{-1}), germanium (10 μg L^{-1}), and scandium (50 μg L^{-1}) were selected as internal standards.Trueness of the bioavailability study was assessed using a mass-balance approach.

Non-dialyzable fractions (residues after the in vitro dialyzability procedure) were subjected to a microwave-assisted acid digestion procedure consisting of adding 4 mL of ultrapure nitric acid and 2 mL of hydrogen peroxide and perform a four-stage microwave irradiation program (power 1000 W). After cooling, the acid digests were diluted to 125 mL with ultrapure water; each sample was analyzed in duplicate and analyzed in triplicate. Two blanks were prepared in each microwave acid digestion set.

5.3. Results and Discussion

5.3.1. Total metal concentration in wine samples

Results of elements concentration in wine are given in Table 5- 1. The content of metals varied considerably among the wine samples (see SFigure 5- 1). Generally the highest contents of metals were found in Pinot noir wines (the highest contents of B, Mg, Al, Ca, Ti, V, Cr, Co, Ni and Sr), Cinsault (the highest content of Li, Fe, Cu, Sn, Sb, Ba, Pt, and Tl), Cabernet Sauvignon (the highest content of Be, Na, K, Mn, and Pb), Muscat of Alexandria (the highest content of Co, Zn and Cd),and Malbec (the highest contents of Tl).

The lowest mineral contents (generally) were found. In Cinsault (the lowest contents of Na, Al, Ca, Ti, Ni, Sr and Cd), Pinot noir (the lowest contents of Cu, Mo, Ba, Hg and Pb), Malbec (the lowest contents of Mn and As), Muscat of Alexandria (the lowest contents of Cr and Fe), Cabernet Sauvignon (the lowest contents of Zn and Tl) and the levels for the Carmenere remained within the ranges for high and low values.

The lowest values of Li, Be, Na, Se, Ag, Sn and Sb were found in Malbec and Pinot noir; whereas, the lowest values of V and Co were were observed in Cinsault and Cabernet Sauvignon. The differences reflect the specific plant genetic capacity to accumulate minerals as well as the soil composition and environmental conditions, cultivation practices, maturity degrees of nuts, and the possible exogenous contributions from sample contamination during shelling and packaging [16, 17].

Elements such as As, Se and Ag were found/detected in some wine samples (levels higher than LOQs). As was found in Carmenere and Pinot noir wines, and Ag in Cabernet Sauvignon.

5.3.2. Bioavailable metal content in wine samples

Metal bioavailability ratios, expressed as a percentage, were calculated using the following equation:

$$Bav(\%) = \frac{[M]_{\text{dialyzate extract}}}{[M]_{\text{total}}} \times 100$$

Where Bav(%) is the percentage of metal bioavailability, and [M]dialyzate extract and [M]acid digest are the metal concentrations after the in vitro digestion procedure, and the microwave assisted acid digestion method, respectively.

Moderate dialyzability ratios were assessed in most wines (fifteen red wines, and three white wines). Li bioavailability was within the 38-77% range; whereas, Ba and B bioavailability ratios were from 21% to 58% (Ba), and from 31% to 50% (B). Mn dializability varied from 17% to 27%, and ratios from 9% to 30% were found for Sr. Major metals, such as Mg, Ca and K, showed ratios within the 16-27%, 11-36%, and 27-43% ranges, respectively (see Figure 5- 1).

Low bioavailability was observed for Al (from 3% to 11%), and for Fe (from 1% to 17%). Regarding Cu, Co, and Sn, most of the dialyzates contained un-detectable concentrations.

Only one wine sample offered a dialyzability ratio of 36% for Cu; whereas Co was quantified in two dialyzates (16 and 18% of dialyzability). Sn was present in eight dialyzates (bioavailability ratios from 5 to 65%). Finally, metals such as Ag, As, Be, Cd, Cr, Hg, Mo, Ni, Pb, Pt, Se, Sb, Ti, Tl, V, and Zn, were not bioavailable (the concentration in dialyzates was lower than the limit of detection of the methods).

Table 5- 2 shows that metal bioavailability percentages varied considerably among different grape samples. The different nutritional composition of wine samples may explain those bioavailability differences. Generally, the highest percentages were found in Cinsault, Muscat of Alexandria and Carmenere wines [the highest contents of bulk biological elements (K, Mg and Ca) and essential trace element (B, Fe, Mn, Co, Cu, Sr, Ba, Sn) metals and non essential (Li and Al)].

This fact may be attributed to their polyphenols content, these inhibitory agents by forming strong chelates and complexes with micro and macro-elements [15]. High percentages were also assessed for Carmenere [High mean value for Li (79.25%), B (48.3%), Mn (25%), Co (16%) and Sn (51%)], Cabernet Sauvignon [Mg (21%) and Sn (65%)] and Muscat of Alexandria [K (38%), Fe Ca (21%), Fe (8%), Co (18), Sr (26%), Ba (59%)] were found see STable 5- 3.

5.3.3. Mass balance

Trueness of the current bioavailability study was assessed using a mass-balance approach using wine samples. Metal concentrations as a sum of the metal concentrations in the dialyzable and non-dialyzable fractions are also listed in STable 5- 5. The mass balance study was performed for each metal by a statistical comparison between the metal concentration as the sum of the metal concentration in the dialyzable and non-dialyzable fractions, and total metal levels in the samples STable 5- 3.

After applying the Cochran's C and Bartlett's tests for variance (standard deviation) statistical comparison of both concentrations (total metal concentration in the sample, and metal concentrations as a sum of metal concentrations in the dialyzate and the nondialyzable fractions) the ANOVA test was performed to compare means for all elements. ANOVA results showed that there were no statistically significant differences between total metal concentration and metal concentration as a sum of metal concentrations in the dialyzate and the non-dialyzable fraction [p-values higher than 0.05 (95% confidence interval)].

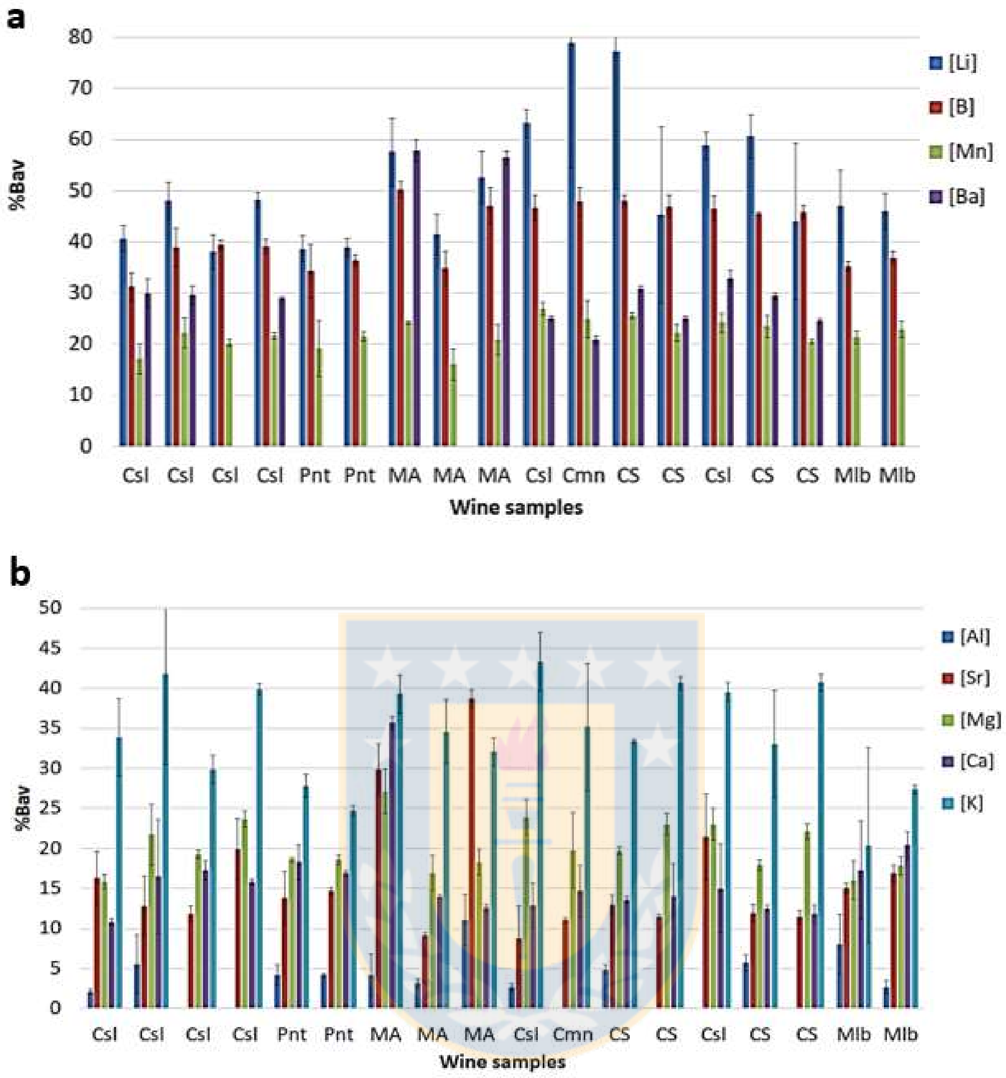

Figure 5- 1. a. Li, B, Mn and Ba, and b. Al, Sr. Mg. Ca and K Bioavailability (%Bav) of in wine samples

Table 5- 1. Total metal concentrations of wine samples from Itata valley.

	Cinsault	**Cabernet S**	**Carmenere**	**Muscat** [a]	**Malbec**	**Pinot**
Li	9 ± 0.1	7 ± 0.1	6 ± 0.1	5 ± 0.1	2 ± 0.1	2 ± 0.04
Be	0.3 ± 0.02	0.4 ± 0.03	0.3 ± 0.03	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.02
B	2957 ± 18	4536 ± 53	6995 ± 156	5036 ± 78	6724 ± 86	15691 ± 291
Na	6268 ± 122	22029 ± 293	20466 ± 425	13586 ± 230	3362 ± 33	3685 ± 50
Mg	101418 ± 16662	130390 ± 1965	141128 ± 2132	116763 ± 1452	178573 ± 1702	256193 ± 4456
Al	345 ± 7	307 ± 7	405 ± 7	393 ± 9	375 ± 4	982 ± 29
K	690 ± 22	863 ± 14	688 ± 12	574 ± 9	478 ± 7	532 ± 8
Ca	6167 ± 94	6978 ± 117	6775 ± 116	6673 ± 79	8438 ± 63	11211 ± 110
Ti	285 ± 5	292 ± 3	396 ± 7	317 ± 4	650 ± 6	844 ± 2
V	1± 0.1	1 ± 0.1	2 ± 0.1	3 ± 0.1	3 ± 0.1	5 ± 0.2
Cr	595 ± 8	583 ± 9	501 ± 12	457 ± 10	624 ± 3	634 ± 10
Fe	1868 ± 25	1093 ± 19	1084 ± 16	1076 ± 96	1139 ± 33	1502 ± 39
Mn	2792 ± 18	2830 ± 17	2782 ± 45	1998 ± 30	1378 ± 19	2207 ± 19
Co	2 ± 0.04	2 ± 0.1	3 ± 0.09	4 ± 0.1	3 ± 0.01	4 ± 0.2
Ni	5 ± 0.3	7 ± 0.3	8 ± 0.3	6 ± 0.2	9 ± 0.5	10 ± 0.3
Cu	112 ± 1	73 ± 0.4	49 ± 0.4	60 ± 0.4	23 ± 0.4	11 ± 0.03
Zn	155 ± 6	141 ± 3	209 ± 6	314 ± 6	279 ± 4	306 ± 10
As	< 0,32	< 0,32	2 ± 0.04	< 0,32	0	2 ± 0.2
Se	< 1,0	< 1,0	< 1,0	< 1,0	0	0
Sr	628 ± 44	1337 ± 19	1487 ± 29	1026 ± 12	1456 ± 17	2719 ± 23
Mo	1 ± 0.2	1 ± 0	0.5 ± 0.09	0.4 ± 0.06	1 ± 0.1	0.3 ± 0.03
Ag	0.2 ± 0.1	0.2 ± 0.1	<0,11	<0,11	0	0
Cd	0.03 ± 0.01	0.06 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.04 ± 0.03	0.1 ± 0.03
Sn	3 ± 1	1 ± 0.3	1.4 ± 0.4	1 ± 0.2	0	0
Sb	3 ± 1	2 ± 1	1.7 ± 0.7	1 ± 0.3	0	0
Ba	285 ± 2	207 ± 1	164 ± 3	104 ± 2	92 ± 1	51 ± 0.4
Pt	0.2 ±.04	0.1 ± 0.04	< 0,011	<0,036	0	0
Hg	< 0,16	< 0,16	< 0,16	< 0,16	< 0,16	0
Tl	0.4 ± 0.01	0.1 ± 0.0	0.2 ± 0.006	0.2 ± 0.01	0.4 ± 0.003	0.2 ± 0.01
Pb	2 ± 0.04	5 ± 0.1	4 ± 0.07	4 ± 0.08	2 ± 0.04	1 ± 0.04

[a] Muscat of Alexandria.

Table 5- 2. Metal concentrations (μg L $^{-1}$) expressed as mean value ± SD in wine samples: 1, 2 Cinsault Yamil Neira; 3, Malbec Chillan, 4, Cinsault J Neira; 5, 6 Pinot Noir Chillan, 7, 8 Muscat of Alexandria, Entre Valles, 9, Muscat of Alexandria, Piedras; 10, Cinsault Entre valles, 11, Carmenere Piedras del E; 12, Cabernet Sauvignon Yneira; 13, Cabernet Sauvignon Entre Valles; 14, Cinsault J Neira; 15, Cabernet Sauvignon Yneira; 16, Cabernet Sauvignon Entre Valles and 17, 18, Carmenere chillan. Before and after in vitro digestion and dialysis step and ICP-MS determination (in vitro dialysis – ICP-MS).

	Li			**B**		
	Bav	Total	%Bav	Bav	Total	%Bav
1	5.1 ± 0.3	13 ± 0.2	41 ± 3	1128 ± 97	3610 ± 30	31 ± 3
2	4.8 ± 0.4	10 ± 0.1	48 ± 4	1189 ± 118	3055 ± 1	39 ± 4
3	0.9 ± 0.1	2 ± 0.1	38 ± 3	2654 ± 43	6724 ± 86	39 ± 1
4	3.6 ± 0.1	8 ± 0.2	48 ± 2	1153 ± 41	2952 ± 28	39 ± 1
5	0.7 ± 0.04	2 ± 0.1	39 ± 3	5271 ± 800	15384 ± 250	34 ± 5
6	0.7 ± 0.03	2 ± 0.02	39 ± 2	5816 ± 117	15998 ± 331	36 ± 1
7	3.3 ± 0.4	6 ± 0.1	58 ± 7	923 ± 29	1838 ± 14	50 ± 2
8	2.5 ± 0.2	6 ± 0.1	41 ± 4	877 ± 82	2516 ± 31	35 ± 3
9	2.5 ± 0.2	5 ± 0.2	53 ± 5	1205 ± 90	2561 ± 23	47 ± 4
10	4.0 ± 0.1	6 ± 0.2	63 ± 3	1049 ± 54	2247 ± 11	47 ± 2
11	3.4 ± 1.0	4 ± 0.2	79 ± 25	2409 ± 70	5043 ± 267	48 ± 3
12	2.1 ± 0.7	3 ± 0.0	77 ± 27	1530 ± 11	3190 ± 70	48 ± 1
13	4.6 ± 1.7	10 ± 0.1	45 ± 17	3038 ± 135	6476 ± 112	47 ± 2
14	4.3 ± 0.2	7 ± 0.04	59 ± 3	1360 ± 68	2922 ± 22	47 ± 2
15	1.8 ± 0.1	3 ± 0.1	61 ± 4	1541 ± 12	3391 ± 10	45 ± 0.4
16	4.7 ± 1.6	11 ± 0.1	44 ± 15	3053 ± 82	6665 ± 53	46 ± 1
17	0.9 ± 0.1	2 ± 0.01	47 ± 7	5942 ± 129	16868 ± 255	35 ± 1
18	0.9 ± 0.1	2 ± 0.1	46 ± 4	6231 ± 216	16907 ± 111	37 ± 1

	Mg			**Al**		
	Bav	Total	%Bav	Bav	Total	%Bav
1	17086 ± 970	107852 ± 1557	16 ± 1	7 ± 1	326 ± 8	2 ± 0.3
2	22195 ± 3927	102194 ± 610	22 ± 4	17 ± 11	304 ± 6	6 ± 4
3	34424 ± 840	178573 ± 1702	19 ± 1	< 9.63	375 ± 4	
4	23947 ± 989	101299 ± 1276	24 ± 1	< 9.63	339 ± 12	
5	46615 ± 47	250548 ± 2757	19 ± 0.2	40 ± 11	947 ± 46	4 ± 1
6	48624 ± 1146	261838 ± 6155	19 ± 1	42 ± 2	1018 ± 13	4 ± 0.2
7	26465 ± 2670	97497 ± 652	27 ± 3	13 ± 8	304 ± 7	4 ± 3
8	17851 ± 2280	105504 ± 957	17 ± 2	15 ± 3	487 ± 5	3 ± 1
9	17093 ± 1459	93579 ± 2103	18 ± 2	19 ± 5	169 ± 23	11 ± 3
10	22878 ± 1984	95719 ± 2280	24 ± 2	11 ± 2	416 ± 3	3 ± 1
11	23546 ± 5566	119252 ± 3027	20 ± 5	< 9.63	311 ± 10	
12	31235 ± 272	158797 ± 3483	20 ± 0.5	17 ± 2	338 ± 3	5 ± 1
13	25865 ± 1443	112348 ± 1506	23 ± 1	< 9.63	251 ± 7	
14	23047 ± 1884	100025 ± 2587	23 ± 2	< 9.63	341 ± 7	
15	29707 ± 1033	165782 ± 2171	18 ± 1	19 ± 3	342 ± 8	6 ± 1
16	25100 ± 1016	113605 ± 1002	22 ± 1	< 9.63	261 ± 8	
17	41747 ± 6343	260610 ± 1238	16 ± 2	81 ± 38	1013 ± 6	8 ± 4
18	47563 ± 3010	267281 ± 2693	18 ± 1	62 ± 19	1032 ± 17	3 ± 1

	K			**Ca**		
	Bav	Total	%Bav	Bav	Total	%Bav
1	335559 ± 47214	990257 ± 18392	34 ± 5	771 ± 1	7120 ± 242	11 ± 0.4
2	408964 ± 110340	979073 ± 15731	42 ± 11	1162 ± 507	7052 ± 34	16 ± 7
3	142791 ± 8017	478034 ± 6501	30 ± 2	1457 ± 100	8438 ± 63	17 ± 1
4	328920 ± 6240	825461 ± 5146	40 ± 1	887 ± 18	5588 ± 2	16 ± 0.3
5	138596 ± 6613	498477 ± 7862	28 ± 1	2025 ± 242	11076 ± 57	18 ± 2
6	139781 ± 3114	566486 ± 8211	25 ± 1	1913 ± 28	11347 ± 163	17 ± 0.3
7	206662 ± 10630	526289 ± 17073	39 ± 2	2019 ± 41	5653 ± 51	36 ± 1
8	274847 ± 32009	794566 ± 6115	35 ± 4	1251 ± 17	8949 ± 43	14 ± 0.2
9	152812 ± 8170	476382 ± 7083	32 ± 2	910 ± 27	7243 ± 143	13 ± 0.4
10	490880 ± 30476	1133065 ± 66875	43 ± 4	705 ± 155	5461 ± 68	13 ± 3
11	351609 ± 78826	999745 ± 13395	35 ± 8	690 ± 152	4710 ± 129	15 ± 3
12	264109 ± 664	789871 ± 5273	33 ± 0.2	1247 ± 30	9215 ± 207	14 ± 0.4
13	370248 ± 5471	910892 ± 12382	41 ± 1	776 ± 225	5527 ± 29	14 ± 4
14	344704 ± 9612	872089 ± 5139	40 ± 1	844 ± 312	5616 ± 124	15 ± 6
15	250225 ± 50454	756980 ± 19161	33 ± 7	1062 ± 26	8476 ± 205	13 ± 0.4
16	365451 ± 8057	897344 ± 11232	41 ± 1	651 ± 58	5507 ± 50	12 ± 1
17	108725 ± 65143	533840 ± 5514	20 ± 12	1944 ± 681	11228 ± 40	17 ± 6
18	143693 ± 2180	525317 ± 7880	27 ± 1	2336 ± 196	11462 ± 41	20 ± 2

	Fe			**Mn**		
	Bav	Total	%Bav	Bav	Total	%Bav
1	32 ± 11	1319 ± 17	2 ± 1	533 ± 94	3120 ± 15	17 ± 3
2	35 ± 12	1297 ± 13	3 ± 1	554 ± 75	2500 ± 6	22 ± 3
3	11 ± 1	1139 ± 33	1 ± 0.1	278 ± 8	1378 ± 19	20 ± 1
4	30 ± 11	1866 ± 48	2 ± 1	693 ± 21	3204 ± 38	22 ± 1
5	18 ± 5	1468 ± 68	1 ± 0.3	417 ± 119	2179 ± 31	19 ± 5
6	9 ± 1	1537 ± 11	1 ± 0.1	482 ± 20	2237 ± 7	22 ± 1
7	58 ± 7	1194 ± 22	5 ± 1	272 ± 1	1120 ± 10	24 ± 0.2
8	60 ± 6	2602 ± 27	2 ± 0.2	540 ± 100	3370 ± 25	16 ± 3
9	33 ± 4	198 ± 5	17 ± 2	349 ± 48	1677 ± 7	21 ± 3
10	54 ± 22	3035 ± 11	2 ± 1	550 ± 26	2041 ± 10	27 ± 1
11	16 ± 5	508 ± 13	3 ± 1	763 ± 106	3063 ± 134	25 ± 4
12	12 ± 6	1017 ± 16	1 ± 1	1035 ± 23	4056 ± 5	26 ± 1
13	< 8	927 ± 20		321 ± 23	1444 ± 14	22 ± 2
14	36 ± 2	1823 ± 35	2 ± 0.1	747 ± 59	3095 ± 22	24 ± 2
15	1 ± 5	745 ± 15	0.1 ± 1	1014 ± 92	4322 ± 40	23 ± 2
16	10 ± 2	908 ± 18	1 ± 0.2	314 ± 7	1534 ± 9	20 ± 0.5
17	32 ± 14	1507 ± 19	2 ± 1	499 ± 28	2342 ± 22	21 ± 1
18	55 ± 19	1092 ± 771	5 ± 4	534 ± 37	2332 ± 18	23 ± 2

	Co			**Cu**		
	Bav	Total	%Bav	Bav	Total	%Bav
1	< 0.2	1 ± 0.03		< 38	73 ± 0.3	
2	< 0.2	1 ± 0.01		< 38	71 ± 1	
3	< 0.2	3 ± 0.04		< 38	23 ± 0.4	
4	< 0.2	3 ± 0.03		48 ± 9	187 ± 1	26 ± 5
5	< 0.2	4 ± 0.2		< 38	11 ± 0.03	
6	< 0.2	4 ± 0.1		< 38	11 ± 0.04	
7	< 0.2	3 ± 0.1		< 38	2 ± 0.1	
8	< 0.2	7 ± 0.1		< 38	212 ± 1	
9	0.6 ± 0.3	3 ± 0.02	18 ± 10	< 38	43 ± 0.2	
10	< 0.2	3 ± 0.1		< 38	36 ± 0.4	
11	1 ± 0.1	6 ± 0.2	16 ± 1	< 38	11 ± 0.4	
12	< 0.2	1 ± 0.05		< 38	37 ± 0.3	
13	< 0.2	2 ± 0.1		< 38	88 ± 0.4	
14	< 0.2	3 ± 0.1	50 ± 12	< 38	193 ± 0.3	26 ± 6
15	< 0.2	1 ± 0.05		< 38	42 ± 0.5	
16	< 0.2	2 ± 0.1		< 38	88 ± 0.2	
17	< 0.2	4 ± 0.1		< 38	13 ± 0.2	
18	< 0.2	4 ± 0.1		< 38	14 ± 0.2	

	Sr			**Ba**		
	Bav	Total	%Bav	Bav	Total	%Bav
1	79 ± 16	488 ± 3	16 ± 3	78 ± 7	260 ± 1.6	30 ± 3
2	90 ± 4	704 ± 203	13 ± 4	105 ± 6	355 ± 5.8	30 ± 2
3	173 ± 14	1456 ± 17	12 ± 1	< 12	92 ± 0.5	
4	101 ± 19	506 ± 6	20 ± 4	82 ± 0.4	283 ± 0.6	29 ± 0.2
5	370 ± 89	2679 ± 27	14 ± 3	< 12	51 ± 0.3	
6	405 ± 12	2759 ± 19	15 ± 0.4	< 12	50 ± 0.4	
7	84 ± 9	283 ± 2	30 ± 3	38 ± 1	65 ± 1.3	58 ± 2
8	111 ± 5	1212 ± 4	9 ± 0.4	< 12	113 ± 0.5	
9	128 ± 4	329 ± 3	39 ± 1	45 ± 0.7	80 ± 1.5	56 ± 1
10	82 ± 37	931 ± 5	9 ± 4	60 ± 0.9	240 ± 0.4	25 ± 0.4
11	120 ± 0	1084 ± 28	11 ± 0.3	4 ± 0.02	218 ± 8.9	21 ± 1
12	211 ± 20	1629 ± 4	13 ± 1	50 ± 0.9	162 ± 0.8	31 ± 1
13	152 ± 4	1328 ± 17	11 ± 0.3	58 ± 1	231 ± 1.8	25 ± 0.5
14	109 ± 27	509 ± 5	21 ± 5	95 ± 4	288 ± 2.4	33 ± 2
15	203 ± 18	1699 ± 16	12 ± 1	40 ± 0.7	136 ± 2.3	29 ± 1
16	160 ± 12	1403 ± 14	11 ± 1	54 ± 0.8	222 ± 0.1	25 ± 0.4
17	437 ± 17	2892 ± 41	15 ± 1	< 12	50 ± 0.5	
18	489 ± 30	2901 ± 3	17 ± 1	< 12	48 ± 0.3	

	Sn		
	Biodisponible	Total	%Bav
1	< 0.3	1.8 ± 1	
2	< 0.3	2.0 ± 0.3	
3	< 0.3	< 0.6	
4	< 0.3	< 0.6	
5	< 0.3	< 0.6	
6	< 0.3	< 0.6	
7	< 0.3	1 ± 0.1	
8	< 0.3	< 0.6	
9	< 0.7	0.6 ± 0.2	
10	0.3 ± 0.04	4.1 ± 1	5 ± 2
11	0.3 ± 0.04	0.7 ± 0.2	51 ± 14
12	< 0.3	0.6 ± 0.1	
13	< 0.3	0.6 ± 0.1	
14	< 0.3	< 0.6	
15	< 0.3	< 0.6	
16	0.3 ± 0.05	< 0.6	65 ± 14
17	< 0.3	< 0.6	
18	< 0.3	< 0.6	

5.4. Conclusion

An in vitro digestion method for assessing the bioavailability of metals using PIPES as a buffer solution and dialysis membranes has been evaluated. An accurate and sensitive method (ICP-MS) was used to determine the trace element concentrations in the different fractions (total in acid-digested samples, dialyzable and nondialyzable fractions).

The proposed method was applied to study the Li, B, Al, Mg, Ca, K, Mn, Fe, Cu, Co, Ba, Sr and Sn bioavailability in different types of wine samples obtaining dialyzability percentages between 0 and 79%. The highest dialyzability percentages were found for Cinsault, Muscat of Alexandria and Carmenere wines. Inorganic contaminants (As, Cd, and Pb) levels were far below the maximum admitted values established by the regional regulations for all samples ensuring food safety.

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Supplementary Material

STable 5- 1. Operating ICP-MS conditions.

General		
	Radiofrequency power/W	1400
	Sample uptake rate/r.p.m.	3
	Stabilization delay/s	35
	Number of replicates	3
Nebulizer type	Beat impact (cooled spray chamber)	
Gas flows/L min^{-1}	Plasma	13
	Auxiliary	0.8
	Nebulizer	0.9
Ion optics/V		
	Extraction	-125
	Lens 1	-1000
	Lens 2	-80
	Lens 3	-195.3
	Hexapole Bias	-4
	Pole Bias	0.2
	D1	-40.8
	D2	-140
	Hexapole Bias	-4
	Pole Bias	0.2
Torch alignment/mm		
	Horizontal	80
	Vertical	405
	Sampling death	150

Continuation

Mass-to-ratio	
	^{7}Li, ^{9}Bo, ^{11}B, ^{23}Na, ^{24}Mg, ^{27}Al,^{39}K, ^{43}Ca, ^{47}Ti, ^{51}V, ^{52}Cr, ^{54}Fe, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{75}As, ^{77}Se, ^{88}Sr, ^{98}Mo, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{138}Ba, ^{195}Pt, ^{202}Hg and ^{208}Pb.
	Internal standards
	^{54}Sc, ^{72}Ge, ^{89}Y, ^{115}In

STable 5- 2. LOD and LOQ for wines

μg L^{-1}	**LOD**	**LOQ**	**SD**
Li	0.03	0.09	0.003
Be	0.02	0.06	0.001
B	1.71	5.69	0.503
Na[a]	10.13	33.78	0.525
Mg[a]	3.67	12.22	0.020
Al	2.53	8.44	0.045
K[a]	35.10	117.00	1.818
Ca[a]	13.00	0.04	0.010
Ti	0.14	0.46	0.004
V	0.02	0.08	0.007
Cr	0.66	2.21	4.030
Fe	4.49	14.97	0.034
Mn	0.12	0.41	0.059
Co	0.02	0.06	0.014
Ni	0.24	0.79	0.044
Cu	0.81	2.69	0.047
Zn	1.37	4.56	0.072
As	0.10	0.32	0.006
Se	1.04	3.47	0.000
Sr	0.06	0.19	0.008
Mo	0.06	0.19	0.001
Ag	0.11	0.35	0.002
Cd	0.05	0.17	0.000
Sn	0.17	0.56	0.002
Sb	0.22	0.75	0.002
Ba	0.39	1.31	0.054
Pt	0.01	0.04	0.000
Hg	0.16	0.54	0.000
Tl	0.00	0.01	0.000
Pb	0.11	0.37	0.007

STable 5- 3. Mean of bioavailable metal in wine samples by grapes.

	Bav%					
Grapes	Cinsault (n = 6)	Pinot noir (n = 2)	Muscat (n = 3)	Carmenere (n = 1)	Cabernet S (n = 4)	Malbec (n = 2)
Li	49 ± 0.14	39 ± 0.06	51 ± 0.13	79 ± 25	57 ± 16	47 ± 6
B	40 ± 2	35 ± 3	44 ± 3	48 ± 3	47 ± 1	36 ± 1
Mg	21 ± 2	19 ± 1	21 ± 2	21 ± 5	21 ± 1	17 ± 1
Al	4 ± 2	4 ± 1	4 ± 3	–^{a}	6 ± 2	6 ± 3
K	38 ± 4	27 ± 1	36 ± 3	36 ± 8	37 ± 1	24 ± 7
Ca	15 ± 3	18 ± 1	21 ± 1	15 ± 3	13 ± 1	19 ± 4
Fe	2 ± 0.9	1 ± 0.5	8 ± 1	3 ± 1	1 ± 1	4 ± 3
Mn	22 ± 2	21 ± 3	22 ± 2	25 ± 4	23 ± 1.4	22 ± 1.5
Co	–^{a}	–^{a}	18 ± 10	16 ± 1	–^{a}	–^{a}
Cu	26 ± 5	–^{a}	–^{a}	–^{a}	–^{a}	–^{a}
Sr	15 ± 4	–^{a}	26 ± 2	11 ± 0.3	12 ± 0.8	16 ± 1
Ba	30 ± 2	–^{a}	59 ± 1	21 ± 1	–^{a}	–^{a}
Sn	5 ± 2	–^{a}	–^{a}	51 ± 14	65 ± 14	–^{a}

–^{a} < LOQ

STable 5- 4. Total element concentration in wine samples

µg L^{-1}	[Li]	[Be]	[B]	[Na]	[Mg]	[Al]	[K] *
Cinsault	13 ± 0.2	0.2 ± 0.03	3610 ± 30	6728 ± 121	107852 ± 1557	326 ± 8	990 ± 18
Cinsault	10 ± 0.1	0.2 ± 0.02	3055 ± 1	6047 ± 104	102194 ± 610	304 ± 6	979 ± 16
Cinsault	2 ± 0.1	0.1 ± 0.01	6724 ± 86	3362 ± 33	178573 ± 1702	375 ± 4	478 ± 7
Cinsault	8 ± 0.2	0.3 ± 0.01	2952 ± 28	5613 ± 93	101299 ± 1276	339 ± 12	825 ± 5
Pinot noir	2 ± 0.1	0.1 ± 0.01	15384 ± 250	3611 ± 6	250548 ± 2757	947 ± 46	498 ± 8
Pinot noir	2 ± 0.0	0.1 ± 0.02	15998 ± 331	3760 ± 95	261838 ± 6155	1018 ± 13	566 ± 8
Muscat	6 ± 0.1	0.3 ± 0.02	1838 ± 14	21779 ± 158	97497 ± 6512	304 ± 7	526 ± 17
Muscat	6 ± 0.1	0.2 ± 0.02	2516 ± 31	6288 ± 81	105504 ± 957	487 ± 5	795 ± 6
Muscat	5 ± 0.2	0.2 ± 0.02	2561 ± 23	10975 ± 320	93579 ± 2103	169 ± 23	476 ± 7
Cinsault	6 ± 0.2	0.3 ± 0.02	2247 ± 11	7230 ± 189	95719 ± 2280	416 ± 3	1133 ± 67
Carmenere	4 ± 0.2	0.2 ± 0.03	5043 ± 267	4103 ± 81	119252 ± 3027	311 ± 10	1000 ± 13
Cabernet S	3 ± 0.0	0.3 ± 0.02	3190 ± 70	3681 ± 51	158797 ± 3483	338 ± 3	790 ± 5
Cabernet S	10 ± 0.1	0.6 ± 0.03	6476 ± 112	47846 ± 1198	112348 ± 1506	251 ± 7	911 ± 12
Cinsault	7 ± 0.0	0.4 ± 0.03	2922 ± 22	5722 ± 101	100025 ± 2587	341 ± 7	872 ± 5
Cabernet S	3 ± 0.1	0.3 ± 0.03	3391 ± 10	3770 ± 65	165782 ± 2171	342 ± 8	757 ± 19
Cabernet S	11 ± 0.1	0.6 ± 0.04	6665 ± 53	48579 ± 29	113605 ± 1002	261 ± 8	897 ± 11
Malbec	2 ± 0.0	0.1 ± 0.02	16868 ± 255	3695 ± 47	260610 ± 1238	1013 ± 6	534 ± 6
Malbec	2 ± 0.1	0.1 ± 0.01	16907 ± 111	4040 ± 21	267281 ± 2693	1032 ± 17	525 ± 8

- mg L^{-1}

Continuation

μg/L	[Ca]	[Ti]	[V]	[Cr]	[Fe]	[Mn]	[Co]
Cinsault	7120 ± 242	231 ± 2	1 ± 0.03	656 ± 10	1319 ± 17	3120 ± 15	1.3 ± 0.03
Cinsault	7052 ± 34	223 ± 2	1 ± 0.1	602 ± 6	1297 ± 13	2500 ± 6	1.3 ± 0.01
Cinsault	8438 ± 63	650 ± 6	3 ± 0.1	624 ± 3	1139 ± 33	1378 ± 19	2.8 ± 0.04
Cinsault	5588 ± 2	317 ± 8	2 ± 0.04	613 ± 17	1866 ± 48	3204 ± 38	2.9 ± 0.03
Pinot noir	11076 ± 57	832 ± 1	5 ± 0.3	622 ± 16	1468 ± 68	2179 ± 31	3.6 ± 0.24
Pinot noir	11347 ± 163	857 ± 3	5 ± 0.02	647 ± 5	1537 ± 11	2237 ± 7	3.8 ± 0.08
Muscat	5653 ± 51	307 ± 0	3 ± 0.1	425 ± 11	1194 ± 22	1120 ± 10	3.2 ± 0.10
Muscat	8949 ± 43	180 ± 1	5 ± 0.1	494 ± 3	2602 ± 27	3370 ± 25	6.8 ± 0.05
Muscat	7243 ± 143	153 ± 1	2 ± 0.1	530 ± 13	198 ± 5	1677 ± 7	3.4 ± 0.02
Cinsault	5461 ± 68	326 ± 3	1 ± 0.1	494 ± 3	3035 ± 11	2041 ± 10	3.0 ± 0.05
Carmenere	4710 ± 129	301 ± 19	0 ± 0.02	624 ± 24	508 ± 13	3063 ± 134	6.1 ± 0.25
Cabernet S	9215 ± 207	195 ± 3	1 ± 0.04	551 ± 1	1017 ± 16	4056 ± 5	1.0 ± 0.05
Cabernet S	5527 ± 29	387 ± 2	1 ± 0.01	585 ± 7	927 ± 20	1444 ± 14	1.8 ± 0.07
Cinsault	5616 ± 124	327 ± 8	2 ± 0.1	612 ± 4	1823 ± 35	3095 ± 22	2.8 ± 0.07
Cabernet S	8476 ± 205	201 ± 1	1 ± 0.1	570 ± 15	745 ± 15	4322 ± 40	1.1 ± 0.05
Cabernet S	5507 ± 50	393 ± 3	1 ± 0.04	616 ± 14	908 ± 18	1534 ± 9	1.9 ± 0.05
Malbec	11228 ± 40	896 ± 6	6 ± 0.2	658 ± 7	1507 ± 19	2342 ± 22	3.7 ± 0.07
Malbec	11462 ± 41	885 ± 13	6 ± 0.1	675 ± 12	1092 ± 771	2332 ± 18	3.8 ± 0.05

μg L^{-1}	[Ni]	[Cu]	[Zn]	[As]	[Se]	[Sr]	[Mo]
Cinsault	4.8 ± 0.8	73 ± 0.3	93 ± 1	< 0.32	< 1.0	488 ± 3	0.9 ± 0.4
Cinsault	3.8 ± 0.2	71 ± 1.3	83 ± 1	< 0.32	< 1.0	704 ± 203	0.7 ± 0.2
Cinsault	8.6 ± 0.5	23 ± 0.4	279 ± 4	< 0.32	< 1.0	1456 ± 17	0.7 ± 0.1
Cinsault	3.9 ± 0.3	18 ± 1.0	234 ± 20	< 0.32	< 1.0	506 ± 6	0.6 ± 0.3
Pinot noir	9.8 ± 0.4	11 ± 0.03	296 ± 18	1.82 ± 0.2	< 1.0	2679 ± 27	0.3 ± 0.0
Pinot noir	10.4 ± 0.2	11 ± 0.04	316 ± 3	1.74 ± 0.1	< 1.0	2759 ± 19	0.3 ± 0.0
Muscat	5.4 ± 0.2	2 ± 0.1	825 ± 13	< 0.32	< 1.0	283 ± 2	0.9 ± 0.1
Muscat	4.6 ± 0.2	212 ± 1.2	198 ± 1	0.34 ± 0.03	< 1.0	1212 ± 4	0.2 ± 0.0
Muscat	6.5 ± 0.2	43 ± 0.2	286 ± 6	< 0.32	< 1.0	329 ± 3	0.3 ± 0.1
Cinsault	7.5 ± 0.2	36 ± 0.4	120 ± 3	< 0.32	< 1.0	930 ± 5	1.9 ± 0.2
Carmenere	11.4 ± 0.2	11 ± 0.4	367 ± 11	< 0.32	< 1.0	1084 ± 28	0.4 ± 0.0
Cabernet S	6.1 ± 0.3	37 ± 0.3	172 ± 1	< 0.32	< 1.0	1629 ± 4	0.6 ± 0.1
Cabernet S	8.8 ± 0.4	88 ± 0.4	90 ± 2	0.35 ± 0.1	< 1.0	1328 ± 17	0.9 ± 0.0
Cinsault	4.2 ± 0.005	193 ± 0.3	245 ± 3	< 0.32	< 1.0	509 ± 5	0.3 ± 0.0
Cabernet S	6.1 ± 0.1	42 ± 0.5	190 ± 5	< 0.32	< 1.0	1699 ± 16	0.5 ± 0.0
Cabernet S	9.0 ± 0.4	88 ± 0.2	97 ± 4	0.39 ± 0.03	< 1.0	1403 ± 14	0.9 ± 0.1
Malbec	10.7 ± 0.3	13 ± 0.2	301 ± 6	1.80 ± 0.04	< 1.0	2892 ± 41	0.3 ± 0.01
Malbec	10.6 ± 0.2	14 ± 0.2	311 ± 9	1.84 ± 0.1	< 1.0	2901 ± 3	0.3 ± 0.005

Continuation

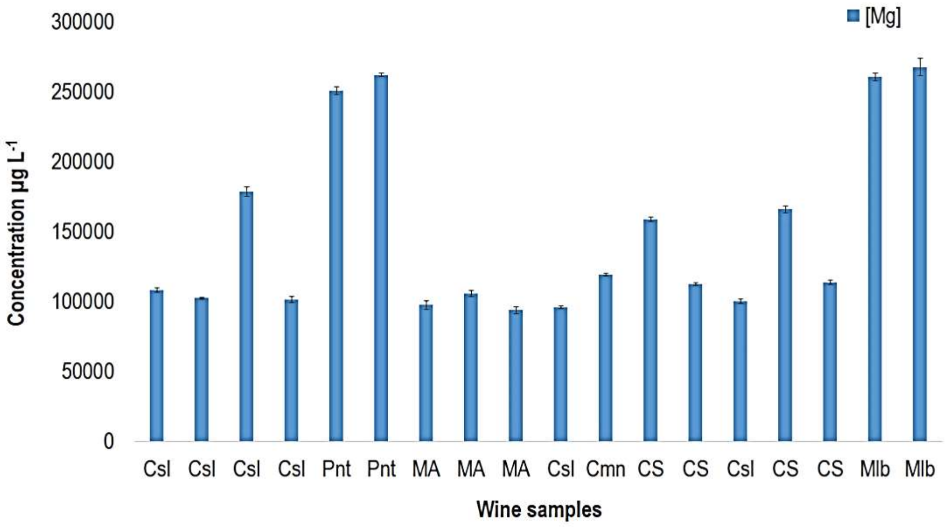
μg L^{-1}	[Ag]	[Cd]	[Sn]	[Sb]	[Ba]	[Pt]	[Hg]
Cinsault	0.203 ± 0.08	0.01 ± 0.01	1.8 ± 0.94	4.8 ± 1.5	260 ± 1.6	0.059 ± 0.03	< 0.16
Cinsault	<0,11	0.01 ± 0.01	2.0 ± 0.30	1.2 ± 0.2	355 ± 5.8	<0.036	< 0.16
Cinsault	<0,11	0.04 ± 0.03	< 0.56	< 0.75	92 ± 0.5	<0.036	< 0.16
Cinsault	<0,11	0.04 ± 0.02	< 0.56	2.9 ± 2.1	28 ± 0.6	<0.036	< 0.16
Pinot noir	<0,11	0.10 ± 0.02	< 0.56	< 0.75	51 ± 0.3	<0.036	< 0.16
Pinot noir	<0,11	0.07 ± 0.04	< 0.56	< 0.75	50 ± 0.4	<0.036	< 0.16
Muscat	<0,11	0.21 ± 0.01	1.1 ± 0.10	0.99 ± 0.1	65 ± 1.3	0.051 ± 0.01	0.207 ± 0.04
Muscat	<0,11	0.03 ± 0.01	< 0.56	< 0.75	113 ± 0.5	<0.036	< 0.16
Muscat	0.128 ± 0.02	0.14 ± 0.01	< 0.56	0.80 ± 0.09	80 ± 1.5	0.047 ± 0.04	< 0.16
Cinsault	0.180 ± 0.10	0.06 ± 0.01	4.1 ± 1.18	2.92 ± 1.2	240 ± 0.4	0.108 ± 0.04	< 0.16
Carmenere	<0,11	0.07 ± 0.01	0.66 ± 0.17	0.74 ± 0.04	218 ± 8.9	<0.036	< 0.16
Cabernet S	<0,11	0.05 ± 0.01	0.65 ± 0.09	1.20 ± 0.4	162 ± 0.8	< 0.011	< 0.16
Cabernet S	<0,11	0.05 ± 0.01	0.59 ± 0.07	< 0.75	231 ± 1.8	< 0.011	< 0.16
Cinsault	<0,11	0.05 ± 0.02	< 0.56	< 0.75	288 ± 2.4	< 0.011	< 0.16
Cabernet S	<0,11	0.07 ± 0.02	< 0.56	< 0.75	136 ± 2.3	< 0.011	< 0.16
Cabernet S	<0,11	0.09 ± 0.02	< 0.56	< 0.75	222 ± 0.1	< 0.011	< 0.16
Malbec	<0,11	0.09 ± 0.04	< 0.56	< 0.75	50 ± 0.5	< 0.011	< 0.16
Malbec	<0,11	0.09 ± 0.01	< 0.56	0.19 ± 0.01	48 ± 0.3	< 0.011	< 0.16

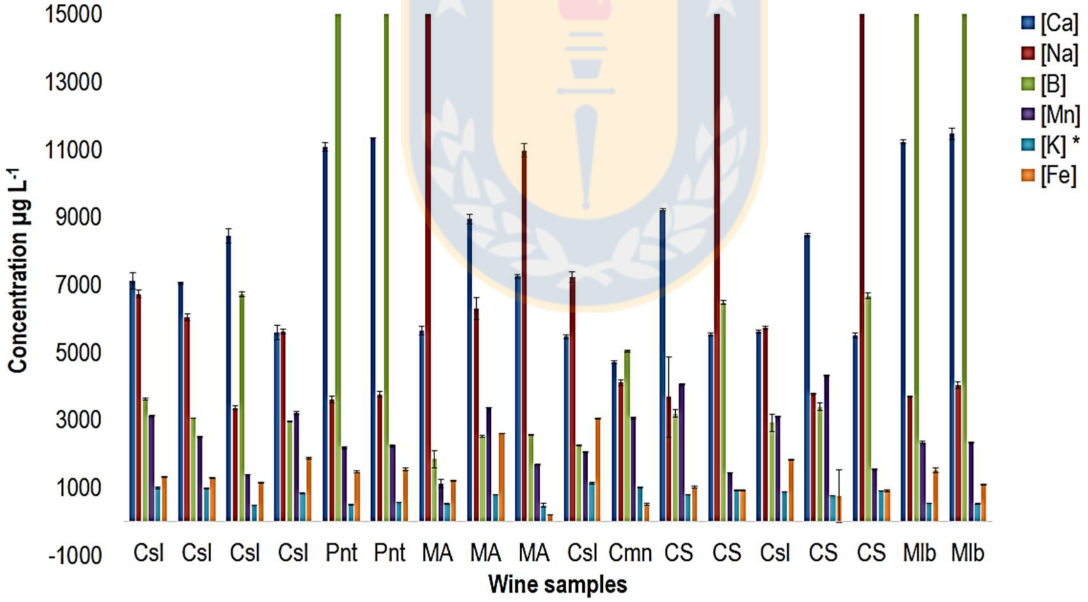
μg L^{-1}	[Tl]	[Pb]
Cinsault	0.2 ± 0.01	1 ± 0.02
Cinsault	0.2 ± 0.004	2 ± 0.02
Cinsault	0.4 ± 0.003	2 ± 0.04
Cinsault	0.2 ± 0.01	2 ± 0.03
Pinot noir	0.2 ± 0.01	1 ± 0.04
Pinot noir	0.2 ± 0.01	1 ± 0.04
Muscat	0.2 ± 0.01	12 ± 0.2
Muscat	0.2 ± 0.01	4 ± 0.1
Muscat	0.3 ± 0.02	1 ± 0.04
Cinsault	0.1 ± 0.004	4 ± 0.04
Carmenere	0.2 ± 0.01	4 ± 0.1
Cabernet S	0.2 ± 0.001	2 ± 0.03
Cabernet S	0.1 ± 0.004	8 ± 0.1
Cinsault	0.2 ± 0.01	2 ± 0.1
Cabernet S	0.1 ± 0.01	2 ± 0.1
Cabernet S	0.1 ± 0.003	8 ± 0.1
Malbec	0.2 ± 0.002	1 ± 0.04
Malbec	0.2 ± 0.01	1 ± 0.1

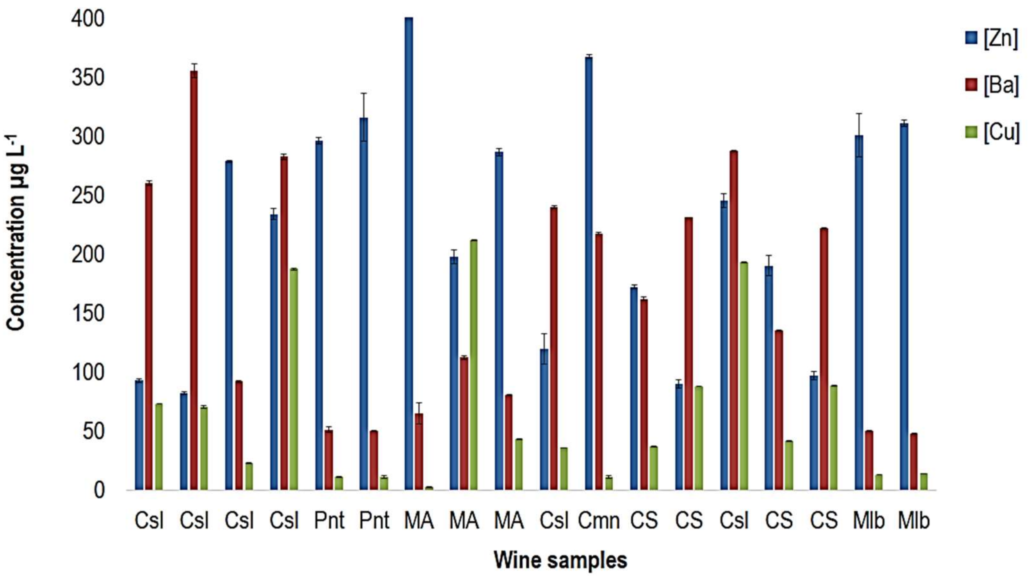
STable 5- 5. Total metal concentrations, dialyzable metal concentrations, and non-dialyzable metal concentrations (expressed as mean value ± SD) in red wine Cinsault (n=3).

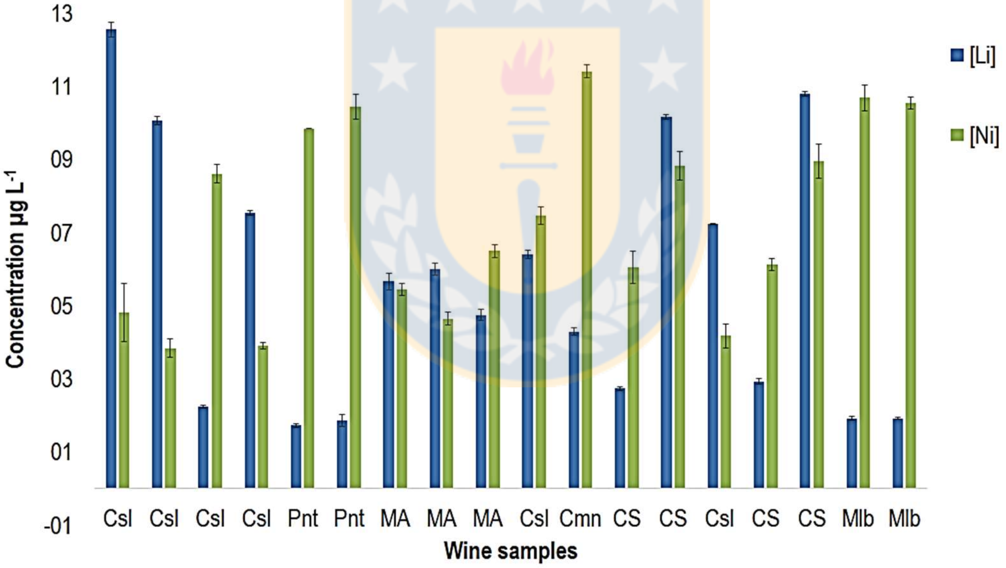
	Total (μg L^{-1})	Total (μg L^{-1})*	Dialyzable (μg L^{-1})	Residue (μg L^{-1})	*p* - value (95%)**
Li	2.24 ± 0.1	2.15 ± 0.1	0.85 ± 0.07	1.30 ± 0.14	0.42
B***	6.72 ± 0.08	6.6 ± 0.07	2.65 ± 0.04	3.9 ± 0.26	0.22
Mn***	1.38 ± 0.02	1.32 ± 0.05	0.28 ± 0.008	1.05 ± 0.04	0.19
Cu	23.2 ± 0.4	22.5 ± 0.7	< LOD	22.5 ± 0.7	0.24
Sr***	1.5 ± 0.02	1.46 ± 0.1	0.17 ± 0.01	1.3 ± 0.131	1.00
Al	375 ± 4	364 ± 17	<LOD	364 ± 17	0.42
Fe	1.14 ± 0.03	1.08 ± 0.1	0.01 ± 0.0001	1.07 ± 0.07	0.51
Co	2.8 ± 0.04	3.52 ± 0.8	<LOD	3.52 ± 0.8	0.28
Ba	92.3 ± 0.05	90.5 ± 11	<LOD	90.5 ± 11	0.84
Mg***	179 ± 2	178 ± 0.05	34 ± 1	143 ± 6	0.18
Ca***	8.40 ± 0.06	8.32 ± 0.09	1.52 ± 0.1	6.86 ± 0.4	0.30
K***	478 ± 7	473 ± 0.06	143 ± 8	330 ± 15	0.08

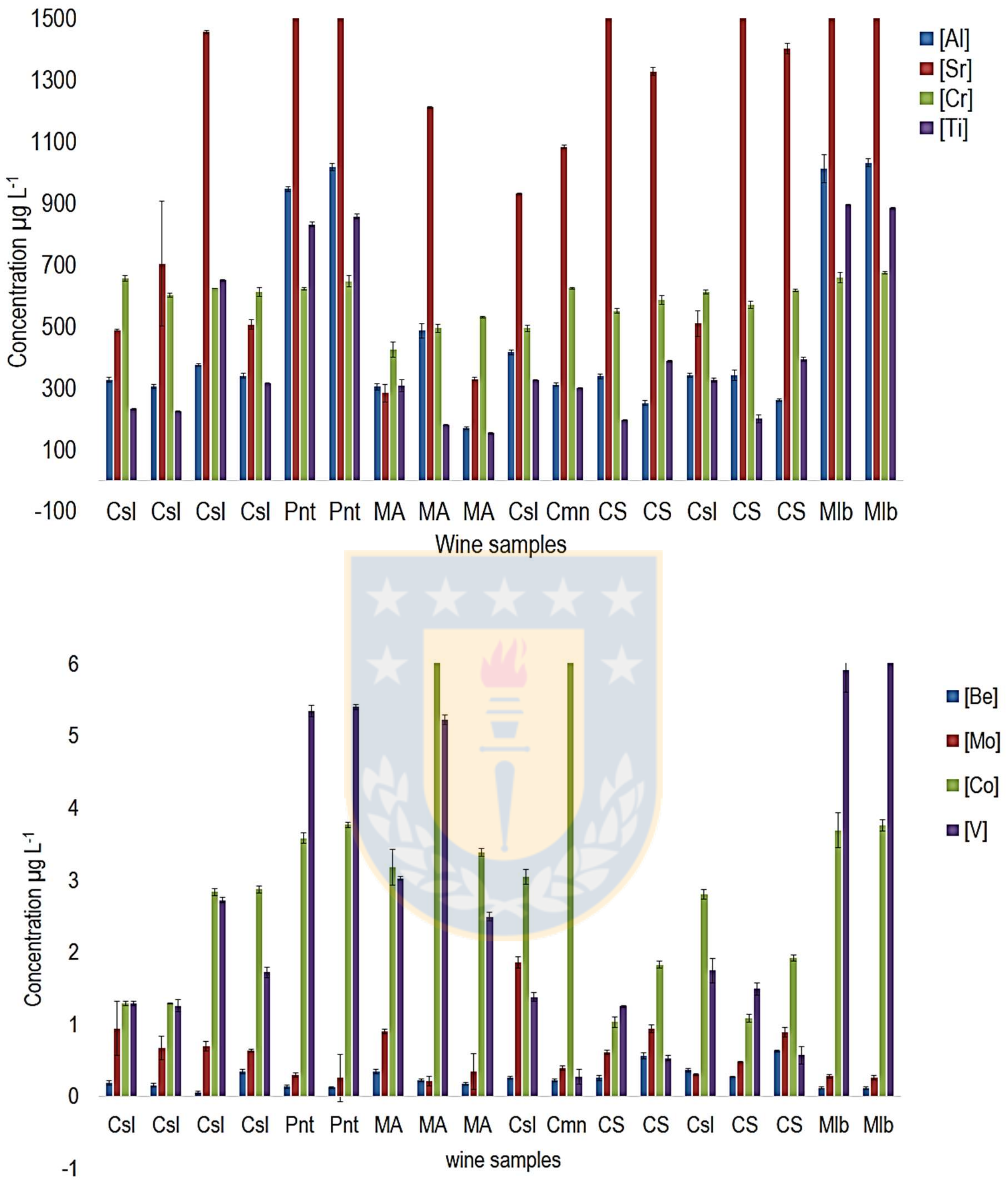
(*) Dialyzable plus residue concentrations; (**) *p*- value after ANOVA; concentrations at mg L^{-1}


[Mg]
300000
250000
200000
150000
100000
50000
0
Concentration µg L-1
Csl Csl Csl Csl Pnt Pnt MA MA MA Csl Cmn CS CS Csl CS CS Mlb Mlb
Wine samples


[Ca]
[Na]
[B]
[Mn]
[K] *
[Fe]
15000
13000
11000
9000
7000
5000
3000
1000
-1000
Concentration µg L-1
Csl Csl Csl Csl Pnt Pnt MA MA MA Csl Cmn CS CS Csl CS CS Mlb Mlb
Wine samples


400
350
300
250
200
150
100
50
0
Concentration µg L-1
Csl Csl Csl Csl Pnt Pnt MA MA MA Csl Cmn CS CS Csl CS CS Mlb Mlb
Wine samples
[Zn]
[Ba]
[Cu]


13
11
09
07
05
03
01
-01
Concentration µg L-1
Csl Csl Csl Csl Pnt Pnt MA MA MA Csl Cmn CS CS Csl CS CS Mlb Mlb
Wine samples
[Li]
[Ni]



SFigure 5- 1. Total metal contents in red (r) and white (w) wines: Csl (Cinsault), Pnt (Pinot noir), MA (muscat of Alexandria), Cmn (Carmenere), CS (Cabernet Sauvignon), Mlb (Malbec).

6. Evaluation for wine by amperometric electronic sensors by chemometrics tools

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Manuscrito en revisión para ser enviado *a Biosensors & Bioelectronics*

Abstract

Twenty-one red wine and eighteen white wine samples from Chile were researched for the use on an array of amperometric sensors and chemometric models tests, to determine the behavior of non-target compounds in wine, what is known as an electronic tongue. Sensors used have been glassy carbon, carbon nanotube and single-walled and multi-walled functionalized electrodes. The potential use of the direct electronic array in the differentiation of wine samples without interfering effects was asses. Nine electrodes were studied by cyclic voltammetry, and five were used for amperometric analysis. The multi dimensionality of the information obtained from the samples was analyzed with chemometric tools. Principal Component Analysis (PCA) and the multi-way decomposition method, parallel factor analysis (PARAFAC), were used as the qualitative modeling tools.

Keywords: electronic tongue; carbon nanotube; chemometric; wine.

6.1. Introduction

According to the IUPAC technical report, electronic tongues defined as "a multisensory system, which consists of some low-selective sensors and uses advanced mathematical procedures for signal processing based on the pattern recognition and multivariate data analysis" (Ha et al., 2015). Therefore, the electronic tongue can be an analytical tool including an array of non-specific, poorly selective chemical sensors with partial specificity (cross selectivity), coupled with chemometric processing (del Valle, 2010).

The most popular sensors in multisensor systems are voltammetric and potentiometric ones (MariaKhaydukova et al. 2017). For recognizing the qualitative and quantitative composition of multispecies solutions. Various sensing methodologies can be used in electronic tongues, such as electrochemical methods (*e.g.,* potentiometry or voltammetry), optical methods, mass change-sensing techniques based on some principles like quartz crystals (Ha et al., 2015), (Lvova, 2016; Wadehra & Patil, 2016). To be suitable for this type of detection, the compound of interest has to be electroactive. This is both a limitation and an advantage: a limitation, as only electroactive species are detectable; an advantage, because the detection can be largely selective. Thus, it is possible to detect electroactive components without the interference of the non-electro active compounds (Scampicchio, Ballabio, Arecchi, Cosio, & Mannino, 2008). Unlike traditional analytical methods, electronic tongue sensors do not provide information on the nature of the compounds under investigation, but only give a digital fingerprint of the food product, which can be subsequently investigated using chemometrics (Scampicchio et al., 2008), (Lvova, 2016).

Chemometric tools can operate the distorted information due to overlapping or interference signals (Scampicchio et al., 2008), (Pigani et al., 2008), (Cetó, González-Calabuig, Capdevila, Puig-Pujol, & del Valle, 2015).

The data-processing algorithms involved mainly include principal component analysis (PCA) which is mostly used in identification/classification for qualitative purpose (Rodríguez-Méndez et al., 2004), partial least squares (PLS) which is mainly used in multi determination for quantitative purpose (Martina et al., 2007), artificial neural networks (ANN) which is a massively parallel computing method, especially suitable for nonlinear sensor signals and extremely related to human pattern recognition (Gallardo et al., 2005). The multi-way decomposition method PARAFAC is a generalization of PCA to higher order arrays, but some of the characteristics of the method are quite different from the ordinary two-way case (Bro, 1997).

Due to some advantages of the electronic sensor such as fast response time, simple automation, less time consuming and operation this system are widely recognized (Scampicchio et al., 2008). Sensor array are used for many analyzes in different types of matrices, agricultural and related areas, pharmaceutical industry, food evaluation, drink discrimination, and even hazards detection, among others (Jun, Guixian, Yong, & Yibin, 2004),(Lvova, 2016), (El Hassani et al., 2018), (Wei, Yang, Wang, Zhang, & Ren, 2018). In the wine analysis, prototypes have been developed for the analysis of food process monitoring, food authenticity assessment, quantitative analysis and quality control studies(Escuder-Gilabert & Peris, 2010). Moreover, materials for manufacturing, the surface of the electrodes can be modified with various chemosensitive materials obtaining sensors of various sensitivity and selectivity towards a variety of species. However, their applicability is limited to redox-active substances (Rodríguez-Méndez, 2001), (Escuder-Gilabert & Peris, 2010).

The aim of the present work is the development of an amperometric sensor based on differently glassy carbon electrodes coupled with data processing techniques to identify different Chilean wine grapes from Itata valley since in our knowledge, this methodology has not been implemented for by local wine producers. The interpretation of complex datasets produced is performed by using multivariate statistics including PCA and PARAFAC analysis. This could be an alternative to the classic analytical methods used so far.

6.2. Materials and methods

6.2.1. Instrumentation

The readings were performed with a potentiostat HCH model 821C. Stirrer instrument. pHmeter and electrochemical cell. The set of working electrodes, Ag / AgCl reference electrode, and an auxiliary platinum electrode were arranged at at room temperature to prepare the analyzes. An ORION 720A plus pH–meter with a glass–calomel electrode (ORION, Cambridge, UK),

6.2.2. Reagents

An electrochemical sensor was developed and optimized to identify grapes of red wine. Mineral oil, BioReagent, for molecular biology, light oil, from Sigma-Aldrich [M5904] (Steinheim, Germany). Non-targeted tests were evaluated on wine samples using an arrangement of nine different electrodes made with mixtures of Glassy Carbon (GC), spherical powder and Single-walled Carbon Nanotube (SWNT) and multi-walled (MWCNT), thin Extent of labeling: >8% carboxylic acid functionalized, avg. Diam. × L

9.5 nm × 1.5 µm. And Carbon nanotube, single-walled, amide functionalized, >90% carbon basis, D × L 4-6 nm × 0.7-1.0 µm, bundle dimensions, suppled by Sigma-Aldrich (Steinheim, Germany). Potassium chloride for analysis EMSURE®. Moreover, high purity ethanol from Pamreac. The deionized water 18.2 MΩ cm, produced by MilliQ Gradient A1, USA.

6.2.3. Wines samples

A collection of forty varietal and young wine samples were obtained directly from the different wine producers and collected of the fermentation tanks before the final process of winemaking and out of alcohol fermentation. The wine samples were collected from the southern wine region in Chile at subregion denominated Itata valley according to with Decree No. 464 local wine legislation and specifically, in vineyards between the Coast, valley, and Andes denominations, geographically located between the west and east. For this study, eighteen white wines (Muscat of Alexandria, Millan and Sauvignon blank) and twenty-one red wines samples and the varieties, Cinsault, Cabernet Sauvignon, Carmenere, Malbec, and Pinot noir, were investigated. Wine samples were collected in polypropylene bottles and stored at 5 °C. The sample analysis measurement was carried out in the first months after of the collection.

Table 6- 1. Materials in electrodes for electrode detection in wines samples analysis.

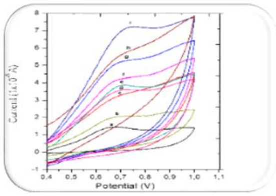
	Material electrode		
n	**1**	**2**	**Composition**
1	GC	-	250 mg GC + 90 µL MO
2	GC	SWNT	250 mg GC + 90 µL MO + 1 mg SWNT
3	GC	carboxylic acid functionalized (SWNT - COOH)	250 mg + 90µL MO + 1 mg SWNT -COOH
4	GC	amide functionalized (SWNT - NH_2)	250 mg + 90 µL MO + 1 mg SWNT -NH_2
5	GC	Graphene	250 mg + 90 µL MO + 1mg Graphene
6	GC	SWNT	250 mg + 90 µL MO + 3 mg SWNT
7	GC	carboxylic acid functionalized (SWNT - COOH)	250 mg + 90 µL MO + 3 mg SWNT - COOH
8	GC	amide functionalized (SWNT - NH_2)	250 mg + 90 µL MO + 3 mg SWNT - NH_2
9	GC	Graphene	250 mg + 90 µL MO + 3 mg Graphene

MO (mineral oil). GC, Carbon glassy spherical powder. SWNT, Carbon nanotube single-walled.

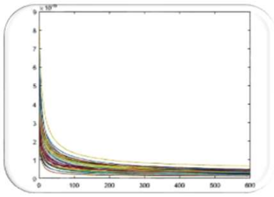
6.2.4. Electrochemical procedures

¡Error! No se encuentra el origen de la referencia.. Displays a scheme of the methodology applied for the elaboration of nine electrodes, optimization of conditions of work and sample analysis used to obtain the data set.

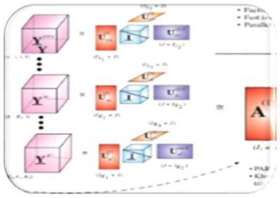
Preparation of electrodes: Nine electrodes were evaluated. Mixes of (SWNT), (SWNT-COOH), (SWNT-NH_2) and grapheme, were prepared with a composition of 1 and 3 mg.



Electrode study by cyclic voltammetry: The conditions for assay were; Scan rate (mV/s) selection (50, 100, 250. Between -0.3 to 1 V versus Ag/AgCl



Amperometric measurement: Work electrodes, GC, GC/SWNT, GC/ 1 mg Graphene and GC/3 mg SWNT. 4 ml (wine sample) + 1ml KCl (0.1M). Init E (V) = 0.69, run time 60s, sample interval 0.1, scan rate 50 mV/s.



Chemometric analysis: With PCA and PARAFAC techniques.

6.2.5. Preparation of electrodes

The paste electrodes of GC, SWNT, SWNT – COOH, SWNT -NH_2 and grapheme were prepared mixed carbon nanotubes and mineral oil with different proportions, 1 and 3 mg. The paste was carefully mixed by hand in a mortar and then packed in a cavity (1 mm in diameter, 5 mm deep) at the end of a Teflon tube. The electrical contact was through a copper wire connected to the paste inside the tube hole. The pulp was kept at room temperature in the dark for 24 hours. The surface of the paste electrodes that were formed was polished on a paper surface before each measurement.

6.2.6. Electrochemical measurements

From cyclic voltammetry and amperometric experiments were developed one electrode at a time. The measurements were done directly on degassed wine samples, without any sample preparation or treatment. Only 1 mL of KCl 0.1 M was added as the electrolyte. Electrochemical experiments were performed using a potentiostat HCH model 821C. An electrochemical analyzer (CH Instruments). The electrochemical cell was assembled with a conventional three-electrode system. Four materials were used for working electrode (1-mm diameter). The Ag|AgCl/KCl (3M) as a reference electrode and Pt counter electrode. All experiments were carried out at room temperature (± 20° C). The conditions for cyclic voltammetry were, the potential was between -0.3 to 1.0 V and the scan rate was studied with 50, 100 and 250 mV s^{-1}. For the amperometric method, the scans were measured at optimized conditions by 60 s. All samples were analyzed in duplicate.

6.2.7. *Data analysis*

For an appropriate treatment of data, the relations between variable and wine grape were identified. The chemometric techniques used in this study were PCA, SIMCA, and PARAFAC. A data matrix with 40 rows (wine samples) and 600 columns (variables) was built. The pattern recognition techniques used in this work were PCA. For the exploration of set data was necessary to distribute the red and white samples in different groups. The PCA methods for exploration in the two groups was used. For multivariate data analysis, the statistical packages Infometrix Inc, with the software Pirouette ver. 4.0, Statgraphics Centurion, version 16.1 (2010) and Matlab were used.

6.3. Results and Discussion

6.3.1. *Optimization of the electrode system*

The amperometric measurements were developed with the optimized conditions obtained in voltammetry's tests. Before to measurements of wine samples by amperometry, the voltage of 0.65 V was chosen as working potential through cyclic voltammetry. In **Figure 6- 1** are detailed of the result of cyclic voltammetry, where an oxidant current was showed with more intensity for electrodes a, b, g and i.

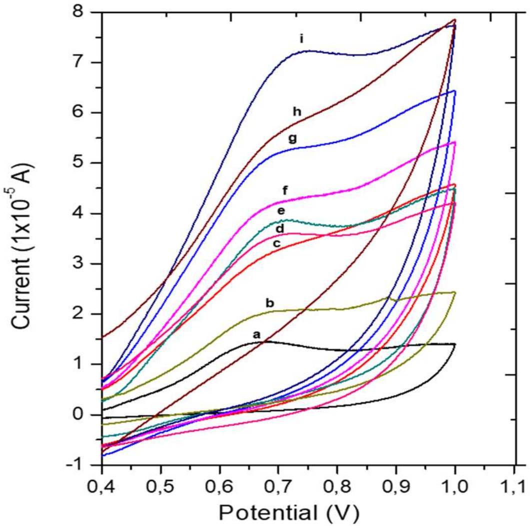


Figure 6- 1. Cyclic voltammetry of wine samples. Optimizing working conditions with 0.1 mol L^{-1} KCl, platinum electrode and working electrode of **a**. GC, **b**. GC/3 mg SWNT, **c.** GC/1 mg SWNT - NH_2, **d.** GC/ 1 mg SWNT, **e.** GC/3 mg Graphene, **f.** GC/1 mg Graphene, **g.** GC/1 mg SWNT - COOH, **h.** GC/3 mg SWNT - NH_2, **i**. GC/3 mg SWNT – COOH were investigated; the scan rate was 50 mV s^{-1} between -0.3 and 1.0 V versus Ag/AgCl.

The electrodes selected with better performance and high signal, the measurements of 40 wine samples were made. The **Figure 6- 2** showed the typically signals obtained for amperometry measurement. In this, for all the readings the potential was constant (0.65V), and current measurements were obtained in a time of 60 s, using the five selected electrodes for the analysis of the 40 wine samples.

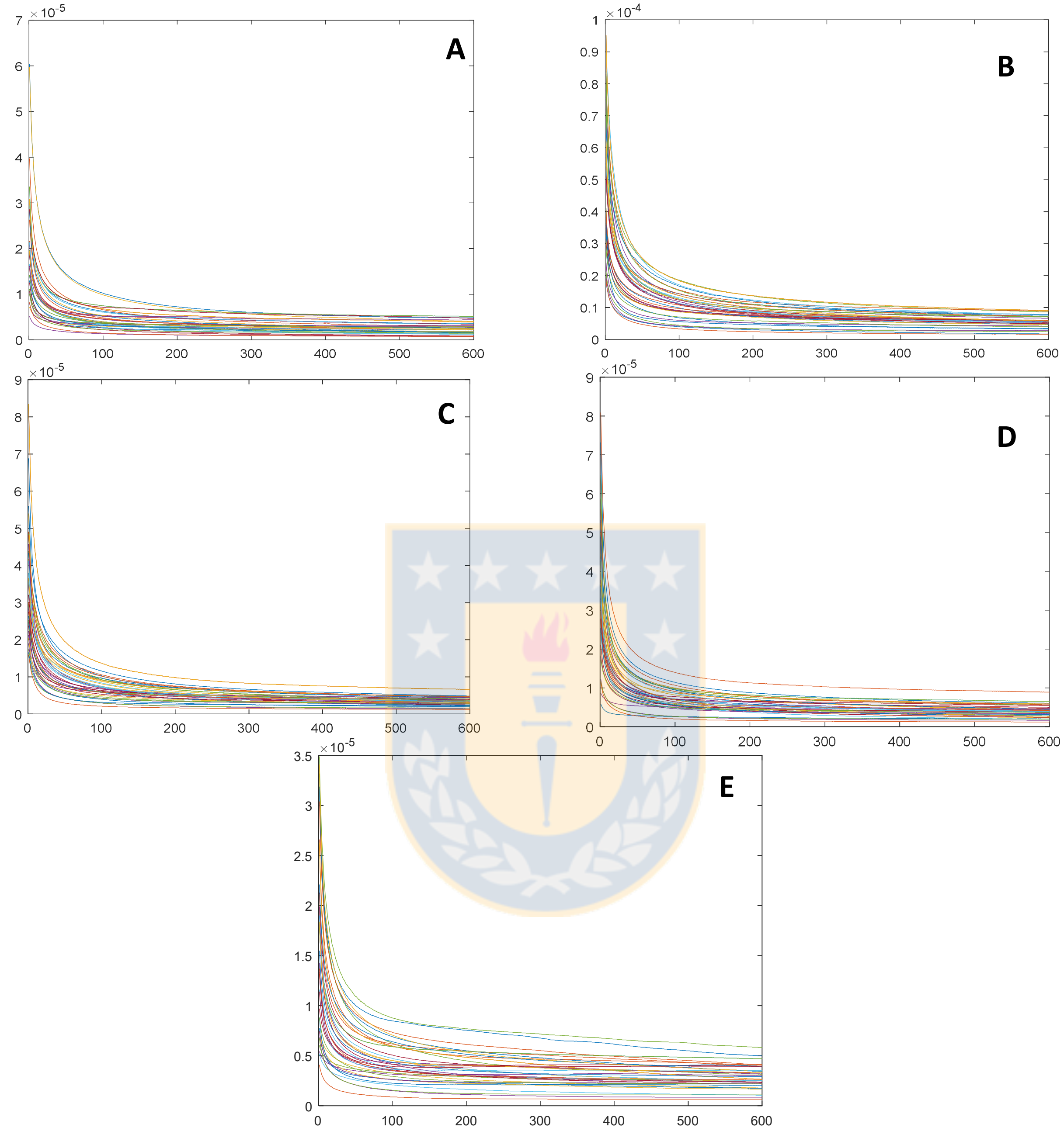


Figure 6- 2. Original data from electrodes array, **a.** GC/ 1 mg CNT, **b.** GC/ Graphene, **c.** GC, **d.** GC/ 3 mg CNT, **e.** GC/ SWNT –COOH.

6.3.2. Chemometric analysis Data analysis

The methodology for exploring data was the PCA, SIMCA, and PARAFAC. The PCA, a procedure that permits to extract useful information from the data, to explore the data structure, the relationship between objects, the relationship between objects and variables and the global correlation of the variables. The six different grapes of wine were investigated with a randomly selected measurement order. For each measurement, 600 values from the amperometric system measurement were collected. The dimensions of the PARAFAC tensor was (40x600x5). A sample with content equivalent to the volume of alcohol in the wine (12%) was treated as a blank sample.

6.3.3. PCA and PARAFAC analysis

From the results and the chemometric analysis, the data were evaluated to determine if the electrode array could classify said samples by grapes and type of wine (white or red). Also, different strategies were developed to improve the percentages of accumulated variance, both for the PCA and PARAFAC and then the results were compared. Among the pre-processing, the scaling and the centering were used. As for the PARAFAC, scaling, centered on the average, centering on the average with scaling and centering on the average with scaling on the variance were used.

Additionally, other types of pre-processing were used for the baseline correction, suggested by (Skov & Bro, 2005), the difference between the maximum signal (or minimum) and the baseline signal, ΔS or S - S0, the fractional approach ($(S(t) - S_0) / S_0$) and relative $S(t) / S_0$ approximation.

For study white and red wines was necessary independently interpretations, since the differences between them produced an increase in the variance of the obtained models, however for the category of the type of wines a separation and results observed in the PCA and PARAFAC models were acceptable.

With this analysis, it is inferred that the electrodes are capable of possibly measuring phenolic compounds, which are those that are mainly found among them, significantly in different proportions. On Figure 6- 3 are represent the scores for the wine samples white and red, with 98.7% of variance accumulated in the two first components. The result of PARAFAC analysis are in **Table 6- 1** showed the result of assays for model without pre-processing, mean center and auto-scaling pre-processing. In which the value of concordance approximated to 100 and lower value in error was selected as the best model for the classification of grapes.

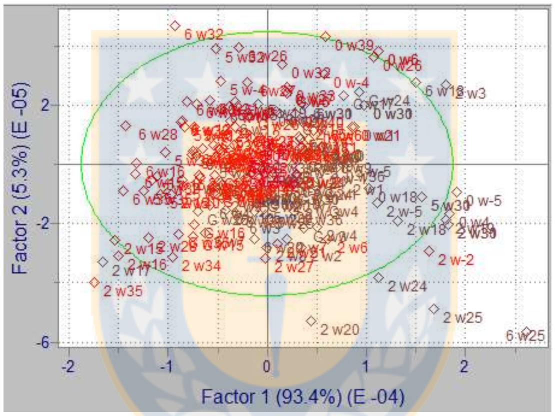


Figure 6- 3. Scores from PCA for wines white and white category.

Table 6- 2. Models by PARAFAC analysis. 1, Data without pre-processing. 2, data with the mean center. 3, Data with the mean center and auto-scaling. 4. Data auto-scaling.

Treatment	Iterations	Concordia	error	Factors
1	97	99.71	1,82x10^{-7}	2
2	56	97.94	1,67x10^{-7}	2
3	26	78.23	3,37x10^{+4}	2
4	242	-4.89	1.37x10^{+4}	3

The red wine grape sample studied were Cinsault, Pinot noir, Cabernet Sauvignon, Malbec, and Carmenere. These samples were collected from Chile and Itata valley. The result from the PCA and PARAFAC were comparable. With a simple amperometric measurement could be differentiated Cinsault wine, with a high percentage of variance explained by PCA and low percentages of error and high concord value by PARAFAC for two component. The mean center pre-processing was enough for these. Carmenere samples were also differentiated, but some of these were mixed with Cabernet Sauvignon grapes. Pinot noir, Malbec and Cabernet Sauvignon were mixed and a good differentiation was not possible. In Figure 6- 4 are represented the different pre-processing performed for the data set for red wines using PARAFAC.

In a first approximation, the data were observed without any treatment, and then the data options were evaluated without the blank difference and mean center, blank difference and data with a blank difference and mean center, which showed the better results. The result of models PARAFAC is detailed in Table 6- 1.

For the white wine the models were studied, the grape Muscat of Alexandria, Sauvignon blanc and Millan were analyzed, and the better result could be improved for the differentiation of grapes Muscatel of Alexandría. However, in this evaluation, it was not possible to make an adequate separation between these grapes.

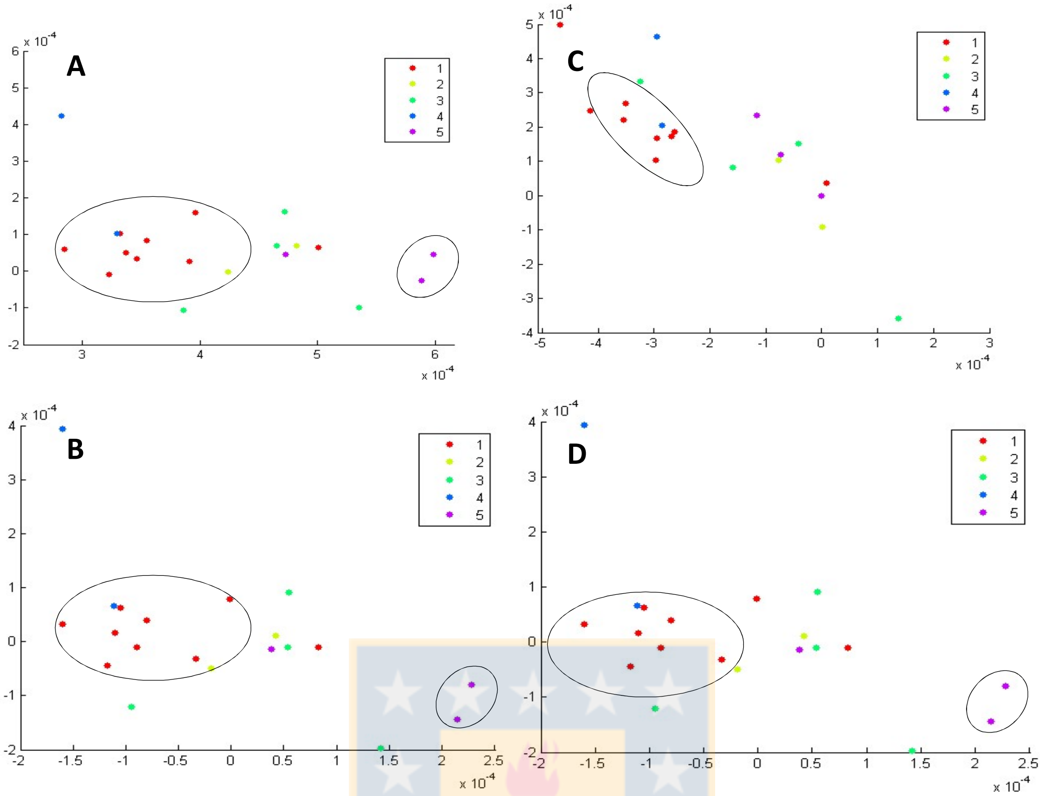


Figure 6- 4. Differentiation of grapes by PARAFAC method, 1 (Cinsault), 2 (Pinot noir), 3 (Cabernet Sauvignon), 4 (Malbec) 5 (Carmenere). A Original data without pre-processing. B, Original data with mean center preprocessing. C, Data with blank difference without pre-processing. D, Data with blank and mean pre-processing.

6.4. Conclusions

The amperometric sensor system with five electrodes of CG in combination with chemometric data processing can be applied for no target assessment of wine samples produced in Itata valley from Chile for differentiation between white and red wine, and the Cinsault and Carmenere grapes. The suggested methodology can be improved by including additional measurements by voltammetry

With this improvement, you could get a very attractive alternative for the traditional procedure since the results from the multisensor system are available within sixty Seconds of measurements, and attained precision in the prediction of grape differentiation. However, much work remains to be done in this field to confirm the broad applicability of the method.

6.5. Acknowledgments

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7. Conclusiones Finales

El método IFS para las determinaciones de ICP-MS mostró ser una alternativa efectiva para reducir la interferencia isobárica en los análisis para muestra de vino. Se obtuvo alta precisión para las determinaciones de Fe, Mn y Zn en vinos con alto y bajo contenido de azúcar residual, mediante el uso de $^{83}\mathrm{Kr}^{+}$ con el método de IFS. Fue la primera vez que se aplicó el método IFS para metales importantes en el vino en una muestra de matriz.

La determinación de la composición inorgánica de muestras de vino de cuatro importantes valles productores de vino del centro y sur de Chile combinados con tratamiento quimiométrico proporciona modelos de clasificación para lograr una diferenciación aceptable del origen geográfico de los denominados "vinos jóvenes y monovarietales".

Con las variables analizadas se consiguieron porcentajes de varianza acumulada aproximadas al 70%. Sin embargo en el análisis exploratorio PCA las relaciones entre las variables y los valles se encuentran altamente superpuestos y aunque en nuestro estudio se mencionan los patrones de separación, la contribución de cada elemento es significativa y, por lo tanto, se requiere un aumento en el número de variables investigadas para determinar claramente los patrones elementales de cada valle y mejorar los modelos de clasificación.

La determinación de los componentes inorgánicos del vino por medio de ICP-MS y la aplicación de diferentes enfoques quimiométricos fueron necesarios para establecer modelos de diferenciación de origen geográfico, enfocado en la orientación este – oeste, de los vinos del valle del Itata. La relación con los grupos y la variable original son considerados. Las muestras que provienen de la costa están orientadas al primer y segundo factor con las variables Ca, Mn, Cr, Fe, Sr y Be. En valle intermedio, las principales variables fueron Na, B, Sb y Ti. Los elementos más importantes para las muestras de los Andes fueron Zn, Al, Mg, P y Cr; por otro lado, para el área de los Andes, las muestras de vino Carmenere mostraron un comportamiento aislado, donde Pb, Cu, Ni y Tl caracterizaron este grupo. Al mismo tiempo, el grupo Cinsault, está conformado por muestras de las diferentes zonas y Li, K, Se y Ba son sus descriptores generales.

Se logró la discriminación de diferentes muestras de vino blanco y tinto del valle de Itata. Los resultados mostraron un alto porcentaje de diferenciación para los modelos, cuando las muestras de vino tinto y blanco fueron estudiadas por separado, siendo el grupo del vino blanco el que presentó los porcentajes de clasificación más altos y las distancias significativas entre categorías. La clasificación según la denotación de la costa, el valle y los Andes genera altos resultados de predicción.

Además de valuar el contenido total de elementos traza y ultratraza en vinos, se estudiaron complejos metálicos de compuestos orgánicos de bajo peso molecular (LMWC), mediante cromatografía bidimensional [(SEC), y (AEC)] hifenada con ICP-MS. Se encontró que elementos tales como B, Cu, Ni, Rb y Ti estaban unidos a compuestos de pesos moleculares dentro del intervalo 200-2000 Da. La fracción SEC fue caracterizada por AEC, en donde se encontraron tres fracciones importantes entre los tiempos 2.6, 3.2 y 3.8 min. La determinación de los elementos de estas fracciones (AEC-ICP-MS) mostró la presencia de B-, Rb- y Li- LMWC. EL B se encontró en la fracción F2; mientras que Li se asoció a la fracción F1. Sin embargo, otros elementos como Rb y Mn, que se asociaron a la fracción SEC, no se observaron después del análisis AEC-ICP-MS. Estos hallazgos podrían implicar alta labilidad de los complejos Rb y Mn-LMWC.

Se ha evaluado un método de digestión in vitro para evaluar la biodisponibilidad de metales usando PIPES como solución tampón y membranas de diálisis. Se utilizó un método preciso y sensible (ICP-MS) para determinar las concentraciones de oligoelementos en las diferentes fracciones (total en muestras digeridas con ácido, fracciones dializables y no dializables). El método propuesto se aplicó para estudiar Li, B, Al, Mg.

La biodisponibilidad de Ca, K, Mn, Fe, Cu, Co, Ba, Sr y Sn se estudió en diferentes tipos de muestras de vino obteniendo porcentajes de dializabilidad entre 0 y 79%. Los porcentajes más altos de diálisis se encontraron para Cinsault, moscatel de Alejandría y vinos de Carmenere. Los niveles de contaminantes inorgánicos (As, Cd y Pb) estuvieron muy por debajo de los valores máximos admitidos establecidos por las regulaciones regionales para todas las muestras que garantizan la inocuidad de los alimentos.

El sistema de sensor amperométrico con cinco electrodos de CG en combinación con procesamiento de datos quimiométricos puede aplicarse para la evaluación no objetivo de muestras de vino producidas en el valle de Itata en Chile para la diferenciación entre el vino blanco y tinto, entre las uvas Cinsault y Carmenere. La metodología sugerida se puede mejorar incluyendo mediciones adicionales por voltametría