SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RAD: 14-23106- -6 **FECHA**: 2015-10-06 09:34:03
DEP: 2020 DIRECCION DE NUEVAS CREACIONES **EVE:** 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II **FOLIOS**: 11
ACT: 519 TRASLADOINFORME

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
JAIME EDUARDO DELGADO VILLEGAS

Asunto: Radicación: 14-23106- -6
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 11935
Folios: 11

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/ES2011/070593				
Fecha de Presentación Internacional	10/08/2011				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado No. 14-023106-00000-0000	4/Febrero/2014
Reivindicaciones:	Radicado No. 14-023106-00000-0000	4/Febrero/2014
	Radicado No. 14-023106-00005-0000	31/Agosto/2015

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Modificaciones aceptadas

Se acepta el nuevo capítulo reivindicatorio (Radicado No. 14-023106-00005-0000) porque no se amplía el objeto de la solicitud.

4. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en

cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso, se tiene en cuenta la totalidad del nuevo capítulo reivindicatorio (Radicado No. 14-023106-00005-0000) para el examen de patentabilidad.

Título de la solicitud: "EXTRACTO DE UVA, COMPLEMENTO NUTRICIONAL QUE LO COMPRENDE Y SU USO COMO INGREDIENTE FUNCIONAL".

El problema planteado en esta solicitud consiste en la necesidad de suministrar un extracto con alto poder antioxidante.

Para resolver este problema técnico la solicitud en estudio proporciona un extracto seco de uva caracterizado porque comprende un porcentaje en peso de compuestos polifenólicos.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 36/87; A23L 1/30; A61P 3/06.

5. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de solicitud internacional: 10 de agosto de 2011 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	US 6,238,673	"Method of producing high flavonol content polyphenol compositions"	29/Mayo/2001
D3	ES2217966	"Productos alimenticios que comprenden un extracto de hollejo, semillas y raspas de uvas"	1/Noviembre/2004
D4	Food Chemestry, Elsevier LTD, NL, vol. 123, no. 4, 15 Diciembre 2010, Págs. 1156-1162	"Chemical characterization an antioxidant evaluation of muscadine grape pomace extract"	Diciembre 2010
D5	Eduardo Pastrana-Bonilla Et al. Journal of agricultural and food chemistry, vol. 51, no. 18, 1 agosto 2003, págs. 5497-5503	"Phenolic content and antioxidant capacity of muscadine grapes"	Agosto 2003

D1[1] divulga un método para la obtención de composiciones de alto contenido de polifenoles, derivados de uvas (Col. 5, líneas 27-37, Col. 7, líneas 19-24 y Ej. 1, Tabla 1).

D3[2] divulga un complemento alimenticio que comprende entre 100 mg y 1 g del extracto de uva (Ej. 3).

D4[3] enseña la composición del extracto de orujo de uva muscadina.

1 www.google.com.co/patents/US6238673
2 https://patentscope.wipo.int/search/en/detail.jsf?docId=es32048354&recNum=1&maxRec=&office=&prevFilter=&sortOption=&queryString=&tab=NationalBiblio
3 http://www.sciencedirect.com/science/article/pii/S0308814610006540

D5[4] divulga en la tabla 1la composición del extracto de uva muscadina.

6. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 1 consiste en una composición de extracto seco de uva CARACTERIZADA PORQUE comprende uno o más compuestos polifenolicos en un porcentaje en peso del total de la composición del extracto comprendido entre 5% y 70% incluidos ambos limites, donde la concentración en peso de compuestos polifenolicos la siguiente:
• Ácido elágico entre 0,01 y 10 *mg/g* de composición del extracto;
• Mirecitina entre 0,01 y 10 *mg/g* de composición del extraclo;
• Quercitina entre 0,01 y 10 *mg/g* de composición del extracto; y
• Flavan-3-oles, entre 0,2 y 25 *mg/g* de composición del extracto, siendo dichos flavan-3-oles:
  • Catequina: entre 0,1 y 10 *mg/g* de composición del extracto;
  • Epicatequina: entre 0,01 y 2 *mg/g* de composición del extracto;
  • Epigalocatequina: entre 0,1y 10 *mg/g* de composición del extracto;
  • Catequina galato: entre 0,001 y 2 *mg/g* de composición del extracto;
y adicionalmente un excipiente, un diluyente o un portador.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D4
composición de extracto seco de uva CARACTERIZADA PORQUE comprende uno o más compuestos polifenolicos en un porcentaje en peso del total de la composición del extracto comprendido entre 5% y 70% incluidos ambos limites, donde la concentración en peso de compuestos polifenolicos la siguiente:	Una composición adecuada para consumo humano que comprende	Extracto de orujo de uva muscadina con una alta concentración de polifenoles
• Ácido elágico entre 0,01 y 10 *mg/g* de composición del extracto; • Mirecitina entre 0,01 y 10 *mg/g* de composición del extraclo; • Quercitina entre 0,01 y 10 *mg/g* de composición del extracto; y • Flavan-3-oles, entre 0,2 y 25 *mg/g* de composición del extracto, siendo dichos flavan-3-oles: • Catequina: entre 0,1 y 10 *mg/g* de composición del extracto; • Epicatequina: entre 0,01 y 2 *mg/g* de composición del extracto; • Epigalocatequina: entre 0,1 y 10 *mg/g* de composición del extracto; • Catequina galato: entre 0,001 y 2 *mg/g* de composición del extracto;	Un extracto de piel de uva que contiene una cantidad sustancial de flavonoles y una alta cantidad de polifenoles: 1.5 mg/L mirecitina, 9.7 mg/L quercitina, 5.2% p/p de flavonol (Ejemplos 1, 2 y 3)	0.62 mg/g de ácido elágico, 0.17mg/g de miriceitna 0.2mg/g de quercetina, Flavan-3-oles: 1.46mg/g de catequina, 1.28mg/g de epicatequina, 0.9mg/g de epigalocatequina (mg/g de extracto) (Tabla 2)
y adicionalmente un excipiente, un diluyente o un portador.	La composición está en forma de cápsulas o tabletas. (Col. 7, línea 43)	No lo divulga.

4 http://pubs.acs.org/doi/abs/10.1021/jf030113c

Los documentos D1 y D4 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque D1 enseña composiciones para administración oral tipo cápsulas o tabletas (Col. 7, línea 43) que comprenden excipientes apropiados como un diluyente o portador y donde dichas composiciones contienen un extracto de uva con un alto contenido de polifenoles que comprende flavan-3-ol, mirecitina y quercetina y el cual contiene mirecitina en una concentración de 1 mg/g, quercitina en una concentración de 6 mg/g y flavan-3-oles en una concentración de 10.2 mg/g (catequina) y 15.5 mg/g (epicatequina) (Ej. 1, Tabla 1). El documento D1 también divulga que este tipo de composiciones tiene propiedades antioxidantes que se pueden utilizar en las industrias alimenticia, cosmética y farmacéutica (Col. 2, líneas 9-11).

La diferencia entre la invención, definida en la reivindicación 1 y el producto de D1 consiste en que el extracto de uva de la presente solicitud contiene ácido elágico en una concentración de 0.01-10 mg/g de composición del extracto.

Estas diferencias no parecen conceder un efecto técnico inesperado o una ventaja de acuerdo con el documento D1, porque es conocido que la uva y el vino comprende compuestos polifenólicos de tipo ácido hidroxibenzoico como el ácido gálico y el ácido siríngico. Los dataos experimentales proporcionados por la solicitante evidencian que el uso de extracto de uva tienen un efecto en la disminución del colestero-LDL y de todos los niveles totales de colesterol, al igual que un incremento en la capacidad antioxidante del plasma, al ser comparada con un grupo placebo (ejemplo 4 y tabla 1). Sin embargo, la solicitud en estudio carece de ejemplos o ensayos que permitan evidenciar un efecto superior con respecto a las composiciones que comprenden extracto de uva divulgados en el arte previo para las que ya era conocido dichos efectos.

El problema técnico objetivo de esta solicitud consiste en la necesidad de composiciones y de un complemento alimenticio alternativo al ya conocido en el documento D1.

Por su parte, el documento D4 enseña (Tabla 2) el extracto de orujo de uva muscadina, el cual comprende 0.62 mg/g de ácido elágico, 0.17mg/g de miriceitna, 0.2mg/g de quercetina, flavan-3-oles como 1.46mg/g de catequina, 1.28mg/g de epicatequina, 0.9mg/g de epigalocatequina (mg/g de extracto). Es evidente que el contenido de compuestos polifenólicos en el extracto por peso seco se encuentra dentro del rango reivindicado de 5-70%.

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría el extracto de orujo de uva de muscadina divulgado en el documento D4 en la composición o el suplemento divulgado en el documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

De manera que la solución propuesta por esta solicitud en la reivindicación 1 y en las reivindicaciones dependientes 2 a 7 es obvia y no tiene nivel inventivo.

La reivindicación 1 consiste en una composición de extracto seco de uva CARACTERIZADA PORQUE comprende uno o más compuestos polifenólicos en un porcentaje en peso del total de la composición del extracto comprendido entre 5% y 70% incluidos ambos limites, donde la concentración en peso de compuestos polifenólicos la siguiente:
• Ácido elágico entre 0,01 y 10 *mg/g* de composición del extracto;
• Mirecitina entre 0,01 y 10 *mg/g* de composición del extraclo;
• Quercitina entre 0,01 y 10 *mg/g* de composición del extracto; y

• Flavan-3-oIes, entre 0,2 y 25 *mg/g* de composición del extracto, siendo dichos flavan-3-oIes:
  • Catequina: entre 0,1 y 10 *mg/g* de composición del extracto;
  • Epicatequina: entre 0,01 y 2 *mg/g* de composición del extracto;
  • Epigalocatequina: entre 0,1y 10 *mg/g* de composición del extracto;
  • Catequina galato: entre 0,001 y 2 *mg/g* de composición del extracto;

y adicionalmente un excipiente, un diluyente o un portador.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D5
composición de extracto seco de uva CARACTERIZADA PORQUE comprende uno o más compuestos polifenólicos en un porcentaje en peso del total de la composición del extracto comprendido entre 5% y 70% incluidos ambos limites, donde la concentración en peso de compuestos polifenólicos la siguiente:	Una composición adecuada para consumo humano que comprende	Extracto de uva muscadina con una alta concentración de polifenoles, que comprende:
• Ácido elágico entre 0,01 y 10 *mg/g* de composición del extracto; • Mirecitina entre 0,01 y 10 *mg/g* de composición del extraclo; • Quercitina entre 0,01 y 10 *mg/g* de composición del extracto; y • Flavan-3-oIes, entre 0,2 y 25 *mg/g* de composición del extracto, siendo dichos flavan-3-oIes: • Catequina: entre 0,1 y 10 *mg/g* de composición del extracto; • Epicatequina: entre 0,01 y 2 *mg/g* de composición del extracto; • Epigalocatequina: entre 0,1 y 10 *mg/g* de composición del extracto; • Catequina galato: entre 0,001 y 2 *mg/g* de composición del extracto;	Un extracto de piel de uva que contiene una cantidad sustancial de flavonoles y una alta cantidad de polifenoles: 1.5 mg/L mirecitina, 9.7 mg/L quercitina, 5.2% p/p de flavonol (Ejemplos 1, 2 y 3)	Por cada 100 g de futa completa fresca y considerando que el contenido de material seco es del 17.4% la fruta contiene: 0.36mg de ácido elágico, 0.36mg de miricetina, 0.22mg de quercetina, 4.1mg de epicatequina, y 4.9mg de catequina. (Tablas 1 y 6, ejemplo Carlos)
y adicionalmente un excipiente, un diluyente o un portador.	La composición está en forma de cápsulas o tabletas. (Col. 7, línea 43)	No lo divulga.

Los documentos D1 y D5 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque D1 enseña composiciones para administración oral tipo cápsulas o tabletas (Col. 7, línea 43) que comprenden excipientes apropiados como un diluyente o portador y donde dichas composiciones contienen un extracto de uva con un alto contenido de polifenoles que comprende flavan-3-ol, mirecitina y quercetina y el cual contiene mirecitina en una concentración de 1 mg/g, quercitina en una concentración de 6 mg/g y flavan-3-oles en una concentración de 10.2 mg/g (catequina) y 15.5 mg/g (epicatequina) (Ej. 1, Tabla 1). El documento D1 también divulga que este tipo de composiciones tiene propiedades antioxidantes que se pueden utilizar en las industrias alimenticia, cosmética y farmacéutica (Col. 2, líneas 9-11).

La diferencia entre la invención, definida en la reivindicación 1 y el producto de D1 consiste en que el extracto de uva de la presente solicitud contiene ácido elágico en una concentración de 0.01-10 mg/g de composición del extracto.

Estas diferencias no parecen conceder un efecto técnico inesperado o una ventaja de acuerdo con el documento D1, porque es conocido que la uva y el vino comprende compuestos polifenólicos de tipo ácido hidroxibenzoico como el ácido gálico y el ácido siríngico. Adicionalmente, la solicitud en estudio carece de ejemplos o ensayos que permitan evidenciar un efecto superior con respecto a las composiciones que comprenden extracto de uva divulgadas en el arte previo y que ya eran una solución al problema técnico.

El problema técnico objetivo de esta solicitud consiste en la necesidad de composiciones y de un complemento alimenticio alternativo al ya conocido en el documento D1.

Por su parte, el documento D5 enseña la composición de 100g de uva muscadina fresca y entera. Si se tiene en consideración que el contenido de sólidos secos de los 100 gramos de uva es de un 17.4%, la fruta contiene 0.36mg de ácido elágico, 0.36mg de miricetina, 0.22mg de quercetina, 4.1mg de epicatequina, y 4.9mg de catequina (Tablas 1 y 6, ejemplo Carlos). Adicionalmente, D5 enseña que los efectos antioxidantes de los polifenoles son ampliamente conocidos (Pág. 5497).

En consecuencia, el la persona del oficio normalmente versada en la materia está en capacidad de adicionar el extracto de uva de muscadina divulgado en el documento D5 en la composición o el suplemento divulgado en el documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

De manera que la solución propuesta por esta solicitud en la reivindicación 1 y en las reivindicaciones dependientes 2 a 7 es obvia y no tiene nivel inventivo.

La reivindicación 8 consiste en un método de obtención del extracto seco de uva partiendo de orujos de uva procedentes de la destilación que comprende las siguientes etapas:

a) Extracción del orujo por difusión continua en contracorriente para obtenerse un orujo de extracción y un jugo de extracción.
b) Prensado del orujo de extracción resultante de la etapa a) obteniéndose un orujo de prensado y un jugo de prensado;
c) mezcla del jugo de extracción obtenido en la etapa a) y el jugo de prensado. obtenido en la etapa b), seguido por enfriamiento de la mezcla resultante a 25°C, depuración de la misma mediante filtración a 100 micras y centrifugación, obteniéndose un jugo clarificado y un residuo sólido;
d) estabilización del jugo clarificado obtenido en la etapa c) mediante adición de alginato sódico;

Características esenciales	D1	D3
Un método de obtención del extracto seco de uva partiendo de orujos de uva procedentes de la destilación que comprende las siguientes etapas:	Un método para la obtención del extracto seco de uva	El extracto natural concentrado de hollejos, semillas y raspas de uva puede obtenerse mediante el siguiente proceso:
a) Extracción del orujo por difusión continúa en contracorriente para obtenerse un orujo de extracción y un jugo de extracción.	Para el orujo de piel de uva, el material es mezclado con un solvente adecuado, preferiblemente alcohol acuoso o agua, que puede ser calentado para acelerar el proceso de extracción. Una	a) Someter a una mezcla de hollejos, semillas y raspas de uva a un proceso de extracción con agua a una temperatura comprendida entre

b) Prensado del orujo de extracción resultante de la etapa a) obteniéndose un orujo de prensado y un jugo de prensado; c) mezcla del jugo de extracción obtenido en la etapa a) y el jugo de prensado. obtenido en la etapa b), seguido por enfriamiento de la mezcla resultante a 25°C, depuración de la misma mediante filtración a 100 micras y centrifugación, obteniéndose un jugo clarificado y un residuo sólido; d) estabilización del jugo clarificado obtenido en la etapa c) mediante adición de alginato sódico;	solución clara es obtenida por decantación estática, centrifugación o filtración (con o sin adición de floculante). El líquido puede ser aplicado a una columna de resina. (Col. 8, líneas 17 a 30)	15°C y 55°C, para obtener un extracto crudo; y b) Separar la fracción que comprende los componentes antioxidantes (polifenoles y antocianos), presente en dicho extracto crudo obtenido en la etapa a), para obtener un extracto concentrado y, opcionalmente, c) Someter al extracto concentrado en la etapa b) a un proceso de desecado o eliminación del solvente con el fin de obtener el extracto concentrado en forma de polvo seco.

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 8 porque enseña un método para la obtención de composiciones de alto contenido de polifenoles, derivados de uvas, que comprende fraccionar los componentes del extracto y secar la fracción recuperada de polifenoles, que comprende flavan-3-ol, mirecitina y quercetina, hasta obtener un producto seco (Col. 5, líneas 27-37, Col. 7, líneas 19-24 y Ej. 1, Tabla 1). El documento D1 también enseña que el método permite la obtención de una composición que comprende compuestos polifenólicos de tipo ácido hidroxibenzoico como el ácido gálico y el ácido siringico (Ej. 1, Tabla 1).

La diferencia entre la invención, definida en la reivindicación 8 y el método del documento D1 consiste en que la anterioridad no divulga las etapas de prensado de orujo y mezcla de los jugos.

Esta diferencia no parece conceder un efecto técnico inesperado o una ventaja al método reclamado porque la solicitud carece de información técnica que evidencie que el método da lugar a un extracto con propiedades mejoradas o que se resuelva un problema relacionado con los métodos de extracción conocidos.

El problema técnico objetivo de esta solicitud consiste en la necesidad de un método alternativo al ya conocido en el documento D1 para la obtención de un extracto de uva.

Por su parte, el documento D3 divulga un proceso para la obtención del extracto natural concentrado a partir de hollejos, semillas y raspas de uva que consiste en las etapas de:

a) Someter a una mezcla de hollejos, semillas y raspas de uva a un proceso de extracción con agua a una temperatura comprendida entre 15°C y 55°C, para obtener un extracto crudo; y
b) Separar la fracción que comprende los componentes antioxidantes (polifenoles y antocianos), presente en dicho extracto crudo obtenido en la etapa a), para obtener un extracto concentrado y, opcionalmente,
c) Someter al extracto concentrado en la etapa b) a un proceso de desecado o

eliminación del solvente con el fin de obtener el extracto concentrado en forma de polvo seco.

El procedimiento de extracción descrito en D3 presenta la ventaja de que no utiliza sustancias químicas ni agentes extraños, por lo que se puede obtener un extracto natural muy puro (concentrado) que no contiene trazas de otros elementos químicos que pudieran ser perjudiciales para la salud.

El procedimiento de D3 permite obtener un extracto que comprende los siguientes compuestos:
Catequina 8.8mg/g
Ácido gálico 2.8mg/g
quercitina 1,2 mg/g
resveratrol 0,42 mg/g (incluye trans- y cis-resveratrol)
malvidina 34,0 mg/g
peonidina 9,0 mg/g petunidina 5,2 mg/g
delfinidina 3,1 mg/g
cianidina 1,0 mg/

Teniendo en cuenta que el documento D1 ya divulgaba un método para la obtención de composiciones de alto contenido de polifenoles, derivados de uvas, que comprende fraccionar los componentes del extracto y secar la fracción recuperada de polifenoles, que comprende flavan-3-ol, mirecitina y quercetina, hasta obtener un producto seco (Col. 5, líneas 27-37, Col. 7, líneas 19-24 y Ej. 1, Tabla 1) y que el documento D3 igualmente enseña un método para obtención de un extracto de uva que comprende compuestos antioxidantes en una elevada concentración, la persona del oficio medianamente versada en la materia habría inferido sin mucho esfuerzo de su parte, métodos de extracción alternativos modificando las condiciones descritas en el documento D1 .

De manera que la solución propuesta por esta solicitud en la reivindicación 8 y en la reivindicación 9 no tiene nivel inventivo.

La reivindicación 10 consiste en un complemento alimenticio caracterizado porque comprende como ingrediente funcional el 100% total de la composición de extracto de uva descrita en cualquiera de las reivindicaciones 1 a 7:

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	**D1**	**D4**
complemento alimenticio caracterizado porque	Una composición adecuada para consumo humano que comprende:	Extracto de orujo de uva muscadina con una alta concentración de polifenoles
comprende como ingrediente funcional el 100% total de la composición de extracto de uva descrita en cualquiera de las reivindicaciones 1 a 7:	Un extracto de piel de uva que contiene una cantidad sustancial de flavonoles y una alta cantidad de polifenoles: 1.5 mg/L mirecitina, 9.7 mg/L quercitina, 5.2% p/p de flavonol (Ejemplos 1, 2 y 3)	0.62 mg/g de ácido elágico, 0.17mg/g de miriceitna 0.2mg/g de quercetina, Flavan-3-oles: 1.46mg/g de catequina, 1.28mg/g de epicatequina, 0.9mg/g de epigalocatequina (mg/g de extracto) (Tabla 2)

Los documentos D1 y D4 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 10 porque D1 enseña composiciones para administración oral tipo cápsulas o tabletas (Col. 7, línea 43) las cuales pueden ser utilizadas como suplementos dietarios, y que comprenden excipientes apropiados

como un diluyente o portador y donde dichas composiciones contienen un extracto de uva con un alto contenido de polifenoles que comprende flavan-3-ol, mirecitina y quercetina y el cual contiene mirecitina en una concentración de 1 mg/g, quercitina en una concentración de 6 mg/g y flavan-3-oles en una concentración de 10.2 mg/g (catequina) y 15.5 mg/g (epicatequina) (Ej. 1, Tabla 1). La composición que comprende flavonoles tiene efecto antioxidante, puede inhibir la oxidación lipoproteínas de baja densidad y puede ser usada en productos cosméticos (Col. 2, líneas 9-11).

La diferencia entre la invención, definida en la reivindicación 10 y el producto de D1 consiste en que la anterioridad no especifica el contenido de ácido elágico en el extracto.

Estas diferencias no parecen conceder un efecto técnico inesperado o una ventaja de acuerdo con el documento D1, porque es conocido que la uva y el vino comprende compuestos polifenólicos de tipo ácido elágico. Adicionalmente, la solicitud en estudio carece de ejemplos o ensayos que permitan evidenciar un efecto superior con respecto a las composiciones que comprenden extracto de uva divulgadas en el arte previo y que ya eran una solución al problema técnico y en particular con el efecto superior derivado de una concentración particular de ácido elágico.

De igual forma, la solicitud carece de información técnica que permita evidenciar la ventaja o el efecto relacionado con la adición de los componentes nutricionales adicionales descritos en la reivindicación 12 de la presente solicitud, por lo cual es evidente que dichos nutrientes tan solo están relacionados con un enriquecimientos del carácter nutritivo de la formulación y actuarán mediante los mecanismos ya conocidos.

El problema técnico objetivo de esta solicitud consiste en la necesidad de un complemento alimenticio alternativo al ya conocido en el documento D1.

Por su parte, el documento D4 enseña (Tabla 2) el extracto de orujo de uva muscadina, el cual comprende 0.62 mg/g de ácido elágico, 0.17mg/g de miriceitna, 0.2mg/g de quercetina, flavan-3-oles como 1.46mg/g de catequina, 1.28mg/g de epicatequina, 0.9mg/g de epigalocatequina (mg/g de extracto). Es evidente que el contenido de compuestos polifenolicos en el extracto por peso seco se encuentra dentro del rango reivindicado de 5-70%. El documento D4 provee un extracto con un alto contenido de polifenoles, que tiene efectos antioxidantes y puede ser utilizado par aprevenir la enfermedad coronaria.

En consecuencia, el la persona del oficio normalmente versada en la materia está en capacidad de adicionar el extracto de orujo de uva de muscadina divulgado en el documento D4 al suplemento divulgado en el documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

De manera que la solución propuesta por esta solicitud en las reivindicaciones 10 y 11 y las combinaciones de la reivindicación 12 no son inventivas.

7. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US 6,238,673	1 – 12	Nivel inventivo
D3	ES2217966	8 a 9	Nivel inventivo
D4	Food Chemestry, Elsevier LTD, NL, vol. 123, no. 4, 15 Diciembre	1 a 7 y 10 a 12	Nivel inventivo

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
	2010, Págs. 1156-1162		
D5	Eduardo Pastrana-Bonilla Et al. Journal of agricultural and food chemistry, vol. 51, no. 18, 1 agosto 2003, págs. 5497-5503	1 a 7	Nivel inventivo

Respuesta a los argumentos de la solicitante

La solicitante presenta diversos estudios y tablas de resultados en su respuesta e indica que el lote A-042406 que corresponde al producto reivindicado, contiene un contenido elevado de fibra y una composición de polifenoles diferente. Adicionalmente, la solicitante indica que el extracto reduce los niveles de lipoproteínas-colesterol y colesterol total en sangre, y aumenta la capacidad antioxidante en el plasma celular y la concentración de vitamina E. Agrega la solicitante que nuevos ensayos realizados han demostrado que el extracto presenta como nueva característica técnica la propiedad de ser muy estable, incluso en las condiciones del tracto gastrointestinal humano.

Con referencia a los argumentos de la solicitante y a los ensayos y resultados presentados, esta Superintendencia se permite presentar los documentos D4 y D5 los cuales fueron igualmente trasladados en el examen realizado por la Oficina Europea. Como se evidencia en los exámenes de patentabilidad, las composiciones, suplementos y método de obtención del extracto reivindicado carece de nivel inventivo frente a la materia contenida en el arte previo representado por los documentos D1, D3, D4 y D5. Como se estableció en el examen de patentabilidad el documento D4 ya había dado a conocer el extracto de orujo de uva muscadina, el cual comprende 0.62 mg/g de ácido elágico, 0.17mg/g de miriceitna, 0.2mg/g de quercetina, flavan-3-oles como 1.46mg/g de catequina, 1.28mg/g de epicatequina, 0.9mg/g de epigalocatequina (mg/g de extracto). Es evidente que el contenido de compuestos polifenolicos en el extracto por peso seco se encuentra dentro del rango reivindicado de 5-70%. El documento D4 provee un extracto con un alto contenido de polifenoles, que tiene efectos antioxidantes y puede ser utilizado par aprevenir la enfermedad coronaria, y por lo tanto el extracto divulgado en D4 también reduce los niveles de lipoproteínas-colesterol y colesterol total en sangre, y aumenta la capacidad antioxidante en el plasma celular y la concentración de vitamina E.

El documento D5 también provee un extracto con las mismas características del extracto de uva de la presente solicitud Si se tiene en consideración que el contenido de sólidos secos de los 100 gramos de uva es de un 17.4%, la fruta contiene 0.36mg de ácido elágico, 0.36mg de miricetina, 0.22mg de quercetina, 4.1mg de epicatequina, y 4.9mg de catequina (Tablas 1 y 6, ejemplo Carlos). Adicionalmente, D5 enseña que los efectos antioxidantes de los polifenoles son ampliamente conocidos (Pág. 5497). Como se puede observar el extracto divulgado en D5 reúne las mismas características del extracto de la presente solicitud, por lo cual es de esperar que posea las mismas propiedades de la composición objeto de la presente solicitud.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

JESUS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-10-13

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Chemical characterization and antioxidant evaluation of muscadine grape pomace extract

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ABSTRACT

Concentrated muscadine pomace extract was chromatographically analyzed for its individual phenolic, flavonoid, and anthocyanidin compounds. This extract was also characterized regarding its total phenolic content (TPC), total flavonoid content (TFC), antioxidative activities in terms of scavenging DPPH free radicals, reducing ferric, and chelating Fe^{2+}. The TPC of this product was 34.1 ± 1.8 mg of gallic acid equivalents(GAE)/g of extract, and TFC was 3.0 ± 0.3 mg of quercetin equivalents/g of extract. Some phenolic compounds including ellagic acid, gallic acid, (−)-epicatechin, (−)-epigallocatechin, catechin, myricetin, quercetin, and kaempferol were identified. Some anthocyanidins including delphinidin, cyanidin, petunidin, peonidin, and malvidin were also identified in the extract by using a combination of retention and spectral properties on a reverse-phase HPLC–PDA. In addition, 3,3′,4,4′5,5′-hexahydroxystilbene-a resveratrol analogue present in the extract was identified for the first time by LC–MS. The results from this study demonstrate that the muscadine pomace extract is rich of natural antioxidants such as phenolics, flavonoids and anthocyanins, and possesses strong antioxidant properties. Besides, the developed methods can be used for routine quality control of the muscadine products for manufacturing efficiency and consistency.

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1. Introduction

Plants are natural, high efficient bioreactors being able to produce various bioactive secondary metabolites, including natural antioxidants that enable to scavenge free radicals in human bodies and alleviate inflammation, thus have health benefits on prevention of many degenerative and chronic diseases such as coronary heart disease, diabetes, Alzheimer's, cataracts, arthritis and ageing (Miller, Rigelhof, Marquart, Prakash, & Kanter, 2000; Morrissey & O'Brien, 1998). Grape is a rich resource of antioxidants including phenolics, flavonoids, and anthocyanins. United States (USDA, 2008) produced 6105 MT or over 13 billion pounds of grape with an estimated market value of $2.9 billion, making it one of the highest valued fruits in the USA. Muscadine (Vitis rotundifolia) is one of grape species native to the southeastern United States, and traditionally not only eaten fresh, but also used in making wine and jelly. Akoh's research group (Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003; Yi, Fischer, & Akoh, 2005) has identified some individual phenolic acids, flavonoids and anthocyanidins by HPLC in muscadine grapes. Three polyphenolic chemicals, i.e., gallic acid, monomeric catechin and epicatechin, were identified from muscadine seed and skin (Yilmaz & Toledo, 2004). The Talcott research group (Lee, Johnson, & Talcott, 2005; Talcott, Brenes, Pires, & Del Pozo-Insfran, 2003; Talcott & Lee, 2002) also confirmed the presence of ellagic acid, some flavonoids and anthocyanidins in the muscadine grape, wine and juice. Besides, Basha, Musingo, and Colova (2004) reported some polyphenolics in bunch grape wines and muscadine wines.

Though the bioactive compounds of muscadine grape have been the subject of a number of investigations due to their potential health benefits, the bioactive compounds in purple muscadine pomace have not been investigated in any details in the past. Muscadine grape pomace is a winery or grape juice processing by-products, consisting of skin, seed, pulp and residual solid. Its yield is about 20% of the harvested grapes. Increased commercial production of muscadine grape has made grape pomace available for possible commercial utilization. As an example, Paulk Vineyards (Wray, GA) has developed a practical fermentation technology to extract valuable bioactive compounds from Nobel muscadine pomace which can be used as an additive ingredient into food formulation.

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Therefore, it is necessary to know the constitutes of the muscadine extract to ensure the quality of the products. In this study, total phenolics, total flavonoids, chemical profiles of phenolics and anthocyanidins in the muscadine pomace extract were determined. In addition, the contribution of these bioactive compounds to the antioxidative ability of the products was also investigated.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), (+)-catechin (95% purity), (−)-eppigallocatechin, (−)-epicatechin (90% purity), gallic acid (90% purity), ellagic acid (95% purity), myricetin (85% purity), quercetin (98% purity), kaempferol (90% purity), and trans-resveratrol (95% purity), ethylenediaminetetraacetic acid (EDTA), cyanidin chloride, delphinidin chloride, malvidin chloride, petunidin chloride, and peonidin chloride were purchased from Sigma-Aldrich (St. Louis, MO). Ferrous chloride and 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (ferrozine) were purchased from Fluka Chemical Co. (Milwaukee, WI). Acetonitrile, methanol, and water (HPLC grade) were from Fisher Scientific (Suwannee, GA). Muscadine extract concentrate was fermented from the Noble muscadine pomace after juice processing, and was generously provided by the Paulk Vineyards, Muscadine Products Corp in Georgia, USA.

2.2. Solid content determination

Solid content of the muscadine extract was determined following the guidelines of the official AOAC (1990) method 967.03. Precisely weighed muscadine extract was put into an aluminum pan and dried for 16 h in an oven set at 95 °C. After drying, the pans were removed from the oven, allowed to cool in a desiccator, and weighed in triplicate. The dry weight was determined as grams of dry matter per 100 g of the extract.

2.3. Determination of the TPC of the extract

The total phenolic content (TPC) of the muscadine extract was determined using a method described previously (Kim, Chen, Wang, & Rajapakse, 2006). Fifty microlitres of properly diluted muscadin extract was mixed with 450 μL of distilled water and 250 μL of 2 N of Folin–Ciocalteu's reagent. The mixture was incubated at room temperature for 10 min and then centrifuged for 10 min at 2000*g*. The absorbance was then measured using a Spectronic 20 GENESYS spectrometer™ from Fisher Scientific Co. (Fairlawn, NJ, USA) at 735 nm. The standard curve was prepared with gallic acid, and the absorbance was converted to the phenolic content in terms of milligrams of gallic acid equivalence (GAE)/g of muscadine extract.

2.4. Determination of the TFC of the extracts

The total flavonoid content (TFC) of the muscadine extract was determined (Park, Koo, Ikegaki, & Contado, 1997). A mixture was made of 0.5 mL of the properly diluted muscadine extract, 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M aqueous potassium acetate and 4.3 mL of 80% methanol. The mixture was then incubated at room temperature for 40 min before the absorbance was spectrophotometrically measured at 415 nm. The standard curve was prepared with quercetin, and the absorbance was converted to the flavonoid content in terms of mg of quercetin equivalence/g of muscadine extract.

2.5. Identification of individual phenolic and flavonoid by HPLC

After the muscadine extract concentrate was diluted with distilled water (1/9, v/v), two millilitres of the diluted muscadine extract were mixed with 2 mL of 80% methanol in 6 N HCl. The samples were vortexed for 1 min, then placed in a water bath shaker set at 60 °C for 2 h for further acidic hydrolysis of flavonoid glycosides to release corresponding aglycons (Pastrana-Bonilla et al., 2003). The hydrolyzed samples were filtered through a Fisherbrand 0.2 μm PTFE filter and analyzed by a Shimadzu LC-10AT HPLC (Kyoto, Japan),which consisted of a Phenomenex C18 column (250 × 4.6 mm, 5 μm) and a Shimadzu SPD-M10V photodiode array detector (PDA). The mobile phase contained, solvent A, methanol/acetic acid/water (10/0.2/89.8, v/v/v); solvent B, acetonitrile; and solvent C, water. A linear gradient mobile phase was used for phenolic separation: at 0 min, 100% solvent A; at 5 min, 90% solvent A and 10% solvent B; and at 35 min, 30% solvent A and 70% solvent B, with 5 min post-run with 100% solvent C. The sample injection volume was 20 μL, and the flow rate was maintained at 0.6 mL/min. The absorbance of the eluant was scanned from 200 to 500 nm by the PDA. The major phenolics in muscadine pomace extract were identified through comparison with the chemical standards by their individual retention times and characteristic spectra. Quantification was calculated based on external standard curve built for each of the analyzed compounds.

2.6. Identification of the major peak by LC–MS

The major unknown peak was collected repeatedly from the HPLC runnings and further analyzed by LC–MS. The LC–MS analyses were performed on an Agilent HPLC system equipped with a variable wavelength UV–Vis detector, coupled with a Agilent 6100 quadrupole MS system. The UV and mass data were acquired and processed using ChemStation Software. The column and mobile phase used were as same as that described in HPLC analysis. The sample injection volume was 10 μL, and the absorbance of the elute was scanned at 340 nm by UV–Vis detector. MS conditions were as follows: Nubulizer gas (N_2) flow, 8 L/min, fragmentor: 140; m/z acquisition from 100 to 500 amu, 1 scan/sec, positive ionization mode.

2.7. Identification of anthocyanidins by HPLC

One millilitre of appropriately diluted musdadine extract was applied into a C-18 Sep-Pak cartridge (Waters Assoc., Milford, Mass., USA), which was previously activated with 5 mL acidified methanol (0.01% HCl) followed by 5 mL acidified water (0.01% HCl). Sugars, acids, and other water-soluble compounds were eluted with 5 mL of 0.01% aqueous HCl, and anthocyanins were recovered with 5 mL acidified methanol. The methanolic elute was concentrated using a Yamato RE400rotary evaporator (Yamato Scientific Co.) at 35 °C, and 15 mL of 2 N HCl was added. Then the solution was transferred into a container that was flushed with nitrogen to replace air, and sealed. This anthocyanin mixture was heated in a boiling water bath for 30 min (Hong & Wrolstad, 1990; Hosseinian, Li, & Beta, 2008). After cooling in an ice bath, the hydrolysate was applied to the C-18 Sep-Pak cartridge again. The anthocyanidins were recovered with 5 ml of acidified methanol (0.01% HCl) and concentrated to ca. 1 mL, and subjected to the HPLC Photo Diode Array (PDA) analyses. Twenty microlitres of the sample were injected and eluted with a gradient mobile phase consisting of solvent A (phosphoric acid/acetate acid/acetonitrile/water = 1/10/5/84) and solvent B (acetonitrile). The elution was carried out at a flow rate of 1.0 mL/min at an ambient temperature with the following program from 0 to 30% for B in 30 min, then programmed to 40% (B) in 32 min. The anthocynidins were

monitored at 520 nm. Each anthocyanidin was identified by comparing with the retention time and scanned spectra of the individual standard, and quantified based on the corresponding standard curve.

2.8. Measurement of antioxidative capacity

The antioxidant capacity of the muscadine extract was determined by the following three *in vitro* assays: the DPPH free radical scavenging assay, the Fe^{2+} chelating assay, and the reducing ferric assay. The antioxidant BHT was used as a reference for the DPPH free radical scavenging assay and the reducing ferric assay; whereas, EDTA was used as the comparison for the metal chelating assay.

2.9. DPPH· free radical scavenging activity

The DPPH free radical scavenging activity of the muscadine extract was determined as previously reported (Wang, Beckham, Morris, Chen, & Gangemi, 2008), with slight modifications. An aliquot of 0.1 mL of the appropriate diluted muscadine extract, 0.1 mL of methanol, 0.3 mL of tris–HCl (pH 7.4), and 0.5 mL of 0.3 mM of DPPH was combined, shaken vigorously, and incubated in the dark at room temperature for 10 min. The absorbance was then spectrophotometrically measured at 517 nm. The scavenging activity was calculated by the following formula:

$$\text{Scavenging activity}(\%) = \left(1 - \frac{\text{Abs of sample at 517 nm}}{\text{Abs of control at 517 nm}}\right) \times 100\%$$

2.10. Metal chelating

The metal chelating effect of the muscadine extract was determined (Zhang, Wang, Chen, Androulakis, & Wargovich, 2007). A reaction mixture of 600 μL of the appropriate diluted muscadine extract and 40 μL of 2 mM $FeCl_2$ was activated by adding 80 μL of 5 mM ferrozine. After moderate vortex, the reaction mixture was incubated at room temperature for 10 min and then spectrophotometrically measured at 562 nm. The metal chelating effect was determined by the following formula:

$$\text{Chelating effect}(\%) = \left(1 - \frac{\text{Abs of sample at 562 nm}}{\text{Abs of control at 562 nm}}\right) \times 100\%$$

2.11. Reducing Power

The reducing power assay was used to evaluate the antioxidant activity of the extract, with the higher absorbance indicating the greater reducing power. The assay was performed according to the method of Chung, Chang, Chao, Lin, and Chou (2002). A mixture of 0.5 mL of the appropriate diluted muscadine extract and 1 mL of 1% potassium ferricyanide [$K_3Fe(CN_6)$] was incubated at 50 °C for 20 min. One millilitre of 10% trichloroacetic acid was then added and the mixture was centrifuged at 500*g* for 10 min. Then, 1 mL of the supernatant was taken out and mixed with 1 mL of distilled water. After adding 0.2 mL of 0.1% $FeCl_3$, the absorbance was spectrophotometrically measured at 700 nm.

2.12. Statistical analyses

All analyses were performed in triplicate and the data were expressed as means ± standard deviations. All data were subjected to the statistical analyses by using SAS program to examine the least significant difference (LSD) at the 95% confidence level.

3. Results and discussion

3.1. Total phenolics and total flavonoid contents

Production of secondary metabolites is controlled by not only genotype but also the environmental effects. The content of the bioactive compounds in the muscadine may vary between different cultivars, be influenced by locations, harvest time, and the growth environment (Lee & Talcott, 2004). In addition, the content of bioactive compounds was also affected by processing conditions (Amerine & Ough, 1980; Spanos & Wrolstad, 1990a,b). Therefore, it is important and critical to determine the phenolic content and characterize individual bioactive compounds of the muscadine products in order to secure its quality control and explore its potential applications as nutraceuticals or additive ingredients of functional foods. As part of the effort to make functional food from the Noble muscadine pomace extract concentrate, characterization of the chemical profile of the extract was thoroughly conducted in this study.

The concentrate of the Noble muscadine pomace extract contained approximate 30% solid. Using the colorimetric determination, it was found that this muscadine skin/seed extract contained a total phenolics (TPC) of 34.1 ± 1.8 mg of gallic acid equivalents (GAE)/g of extract, and the total flavonoid content (TFC) of 3.0 ± 0.3 mg of quercetin equivalents/g of extract (see Table 1). This muscadine pomace concentrate contains about four folds of the TPC of the muscadine skin/seed powder with 8.8 mg GAE/g of muscadine powder (data analyzed in the same lab), indicating consumption of 2.5 ml of this muscadine pomace concentrate can provide similar amount of antioxidants as 10 g of muscadine skin/seed purple powder.

3.2. Chemical profiles of muscadine extract

3.2.1. Phenolics

Phenolic acids have received considerable attention as potentially protective agents against cancer and heart diseases, in part, attributed to their potent antioxidative and antinflammatory properties. The difficulty of phenolic determination is the extractability of these compounds from food matrix, because phenolic compounds exist in fruits either in the free form or as chemical conjugates (e.g., esterified, and glycolysated phenolic acids). In this study, strong acid hydrolysis was adopted to release the free phenolics from the bound phenolics (Pastrana-Bonilla et al., 2003). The hydrolyzed extract was separated and characterized by a reverse-phase HPLC coupled with a PDA which was successfully applied to the analyses of phenolics in the muscadine pomace extract. Though enzymatic hydrolysis is also feasible (DuPont, Bennett, Mellon, & Williamson, 2002), however, the phenolics from enzymatic hydrolyzed sample needs to be solvent extracted, centrifuged, and filtered prior to HPLC injection. The procedure is more time consuming and more complicated comparing with the direct acid hydrolysis for phenolic acid analyses. So the direct acid hydrolysis method was used in our study.

Fig. 1 shows the chromatogram of phenolics in the muscadine pomace extract. In the Fig. 1A, HPLC–PDA chromatogram indicated the presence of more than 17 phenolic acids eluted within 40 min. As an example, the UV–Vis spectrum of quercetin at 32.556 min is

Table 1
Total phenolic content and total flavonoid content of muscadine pomace extract.

	mg of gallic acid equivalent (GAE)/g of muscadine extract	mg of quercetin equivalent/g of muscadine extract (TFC)
Muscadine extract	34.1 ± 1.8	3.0 ± 0.3

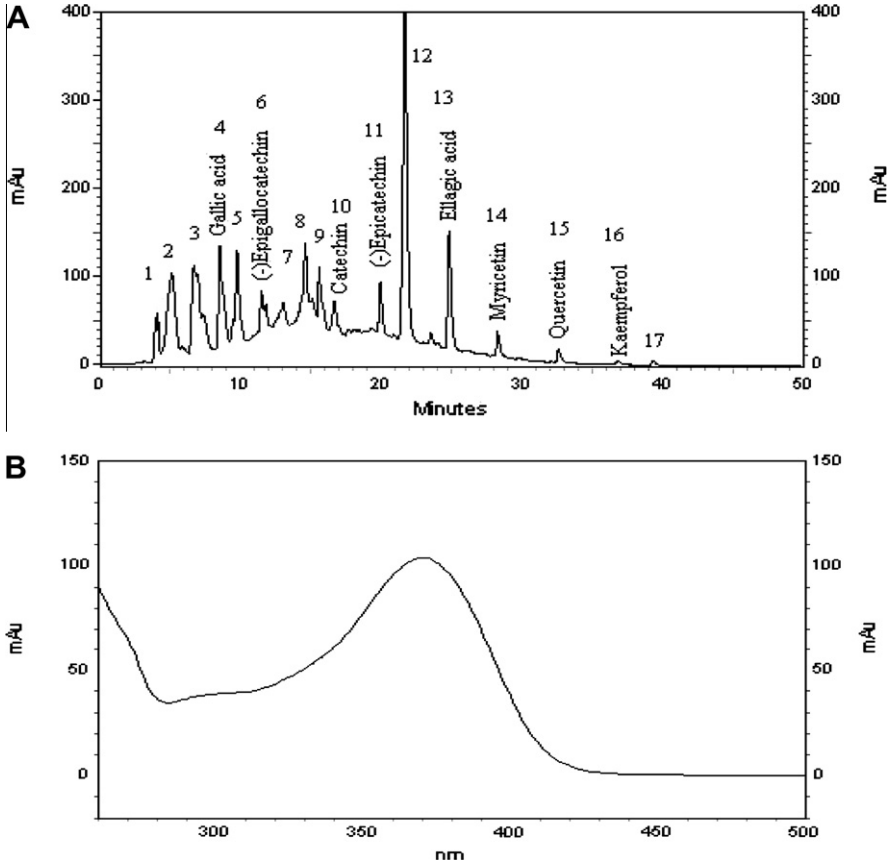

Fig. 1. (A) HPLC chromatogram of muscadine extract at 275 nm. (B). UV–Vis spectrum of peak quercetin at 33.556 min shown in (A).

given in Fig. 1B. In addition, ellagic acid, gallic acid, (−)-epigallocatechin, (−)-epicatechin, catechin, myricetin, quercetin, and kaempferol were all successfully separated and identified by comparing their elution times and spectra with the standards. Each phenolic content in the extract was calculated based on calibration curves of external standards built for each of the analyzed compounds (Table 2). The content of major phenolics detected in the muscadine pomace extract ranged from the highest concentration of 1.46 mg/g for (−)-catechin to the lowest of 0.08 mg/g for kaempferol.

Peak 12 in the HPLC chromatogram (Fig. 1) could not be identified by HPLC–PDA. This compound was repeatedly collected from HPLC running and further analyzed by the LC–MS. Mass spectra of peak 12 is shown in Fig. 2A, from which it was identified as 3,3′,4,4′5,5′-hexahydroxystilbene-a resveratrol (3,4′,5-trihydroxystilbene) analogue. Mass spectrum of the trans-resveratrol is also shown in Fig. 2B. To the best of our knowledge, this is the first report of the presence of the chemical 3,3′,4,4′5,5′-hexahydroxystilbene in the muscadine products. This chemical has shown superior radical scavenging activities (O_2^- and DPPH·) and anti-cancer activity comparing with resveratrol (Murias et al., 2005). The presence of more potent bioactive compound makes the muscadine pomace extract more valuable as nutraceutical ingredient.

Table 2
Concentration of chemicals in muscadine pomace extract (mg/g of muscadine pomace extract).

Compounds	Concentration
Gallic acid	0.25 ± 0.01
(−)-Catechin	1.46 ± 0.21
(−)-Epicatechin	1.28 ± 0.03
(−)-Epigallocatechin	0.9 ± 0.03
Ellagic acid	0.62 ± 0.01
Myricetin	0.17 ± 0.01
Quercetin	0.2 ± 0.0
Kaempferol	0.08 ± 0.01
Delphinidin	5.57 ± 0.05
Cyanidin	3.62 ± 0.04
Petunidin	15.50 ± 0.06
Peonidin	25.32 ± 0.08
Malvidin	10.39 ± 0.06
Pelargonidin	Not detected

Comparison of the chromatographic profiles of muscadine grape extract has provided some clues for a variety of compounds present in the extract. Unfortunately there are relatively few articles available, where chromatographic profiles were presented. In a previous report (Pastrana-Bonilla et al., 2003), eight phenolics were identified in muscadine grape (i.e., ellagic acid, myricetin, quercetin, kaempferol, resveratrol, (−)-epicatechin, (+)-catechin, gallic acid), and four phenolics were identified in the muscadine skin extract, i.e., ellagic acid, myricetin, quercetin, and kaempferol. Later, ellagic acid, resveratrol, myricetin, quercetin, and kaempferol were also identified in muscadine grape by Yi et al. (2005). In our study, we identified the presence of ellagic acid, gallic acid, (−)-epigallocatechin, (−)-epicatechin, catechin, myricetin, quercetin, kaempferol, and 3,3′,4,4′5,5′-hexahydroxystilbene in the muscadine pomace extract, but resverotrol was not detected. The fermentation extraction process may convert the resveratrol into resveratrol analogue, 3,3′,4,4′5,5′-hexahydroxystilbene. This may explain why we found a high amount of resveratrol analogue in the extract instead of resveratrol.

3.2.2. Anthocyanidins

The isolation and quantitation of anthocyanins in grape products is difficult due to their chemical complexity. By using LC–

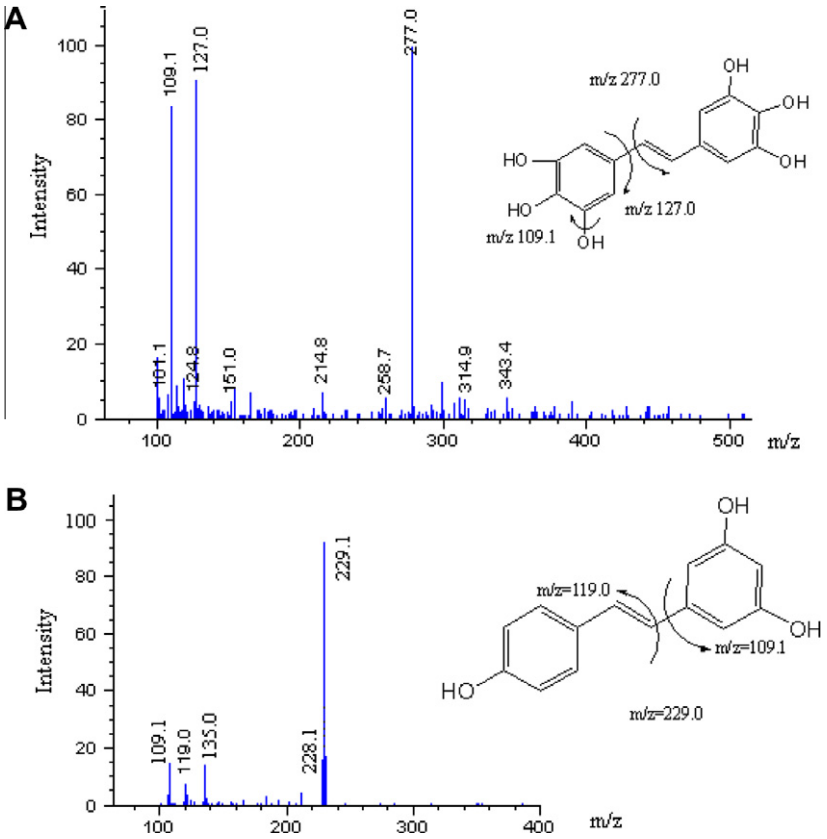

Fig. 2. (A). Mass spectrum of the peak 12 with suggested structure. (B). Mass spectrum of trans-resveratrol with structure.

MS, Talcott et al. (2003) reported the presence of anthocyanins in processed muscadine products: delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, petunidin 3,5-diglucoside, pelargonin 3,5-diglucoside, peonidin 3,5-diglucoside, malvidin 3,5-diglucoside. By using HPLC–MS, Garcia-Beneytez, Cabello, and Revilla (2003) also successfully identified 20 anthocynins: delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, peonidin 3-*O*-acetylglucoside, malvidin 3-*O*-acetylglucoside, malvidin 3-*O*-caffeoylglucoside, cyanidin 3-*O*-*p*-coumarylglucoside, petunidin 3-*O*-*trans*-*p*-coumarylglucoside, peonidin 3-*O*-*cis*-*p*-coumarylglucoside, malvidin 3-*O*-*cis*-*p*-coumarylglucoside, peonidin 3-*O*-*trans*-*p*-coumarylglucoside, malvidin 3-*O*-*trans*-*p*-coumarylglucoside.

Since there are only six common anthocyanidins, identification of the anthocyanidins will make chemical analyses relatively easy. Also, analysis of anthocyanidins is a good initial method in that it yields information of the nature of the aglycones. However, since the heterocycle of anthocyanidins is a pyrilium cation, anthocyanidins are typically not found as free aglycones. These compounds are present in the vacuoles of coloured plant tissues such as leaves, fruit skin, or flower petals. The colour of the pigment depends on the pH, metal ions present, and the combination of substituted

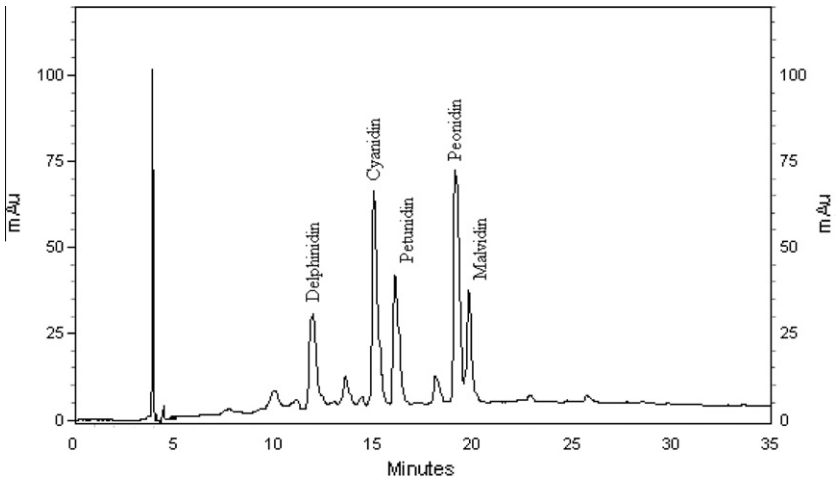

Fig. 3. (A) HPLC chromatogram of anthocyanidins (hydrolyzed anthocynins) from the muscadine pomace extract at 520 nm.

sugars and acyl esters. Anthocyanins are water soluble glycosides of anthocyanidines. Hydrolysis under harsh conditions, such as heating in acid, yields anthocyanidines. In this study, the muscadine pomace extract was firstly purified through C_{18} Sep-Pak cartridges, then hydrolyzed in acid and analyzed by the HPLC–PDA. Five anthocyanidins were identified as delphinidin, cyanidin, petunidin, peonidin, and malvidin (Fig. 3). This extract contains petunidin in amount of 25.3 mg/100 mL, peonidin at 15.50 mg/100 mL, malvidin at 10.39 mg/100 mL, delphinidin at 5.57 mg/100 mL, and cyanidin at 3.62 mg/100 mL (see Table 2).

Hong and Wrolstad (1990) identified cyanidin and peonidin in cranberry, and delphinidin and cyaniding in roselle, as well as pelargonidin as the major pigment with lesser amounts of cyanidin in strawberry. In the muscadine pomace extract, we identified five anthocyanidins: delphinidin, cyanidin, petunidin, peonidin, and malvidin. We did not find pelargonidin in the muscadine pomace extract, which is consistent with the results from previous reports (Garcia-Beneytez et al., 2003; Yi et al., 2005).

3.3. Antioxidative ability of muscadine pomace extract

The muscadine pomace extract contains various phenolics, flavonids and anthocyanins, among which some chemicals show strong antioxidative activities. Among the identified chemicals, gallic acid has been known for its strong antioxidative ability. Ellagic acid is a polyphenolic antioxidant which can be found in raspberries, strawberries, cranberries, and pomegranates. The antiproliferative and antioxidant properties of ellagic acid have also been documented. (−)-Epicatechin and catechin have also been well studied and showed potentials in the prevention of stroke, heart failure, cancer and diabetes. Quercetin also has potential to prevent cancers, heart diseases, inflammations, respiratory disease. In addition, kaempferol, quercetin and myricetin were reported to reduce pancreatic cancer in a clinical trial (Nothlings, Murphy, Wilkens, Henderson, & Kolonel, 2007).

The DPPH scavenging effects of muscadine pomace extract increased with concentration (Fig. 4). At 1/500 dilution, the extract showed similar free radical scavenging activity (about 70%) to BHT at 100 ppm. In another word, 2 mg of the muscadine extract could scavenge same amount of the DPPH free radicals as 0.1 mg of BHT. The presence of free radicals may damage biological components such as proteins, lipids, nucleic acids (RNA and DNA), thus trigger many degenerative and chronic diseases. Reactive oxygen species (ROS), such as superoxide and nitric oxide, and the natural byproducts of the normal metabolism of oxygen, play important roles in cell signaling. However, upon over stressed environment, ROS levels can increase dramatically, which could damage cell structures. The presence of antioxidants can quench these free radicals and alleviate the oxidative stress.

In this reducing power assay, the presence of reducers (i.e., muscadine extract) converted the Fe^{3+}/ferricyanide complex present in the assay system to the ferrous form. By measuring the absorbance at 700 nm to determine the Fe^{2+} concentration, it is possible to estimate the reducing powder of the reducers. The reducing power of the muscadine pomace extract increased along with the concentration (Fig. 4). Muscadine extract at 1/2000 dilution had similar reducing power (about 0.42 at 700 nm) as 100 ppm of BHT, and 1/500 diluted muscadine extract had reducing power at 0.92.

The metal chelating effect of muscadine extract was also dose-dependent. In this assay, the muscadine extract disrupted the ferrozine–Fe^{2+} complex, thus decreased the red colour. The measurement at 562 nm of colour reduction was used to estimate the chelating activity (Fig. 4). Muscadine extract at 1/500 dilution exhibited similar metal chelating activity (35–38%) of 50 ppm of EDTA. Iron is essential to nearly all known organisms, however, excessive iron can be toxic, because free ferrous ions react with peroxides to produce free radicals, which can damage some biological moleculars, such as DNA, proteins, lipids, and other cellular components. The metal chelating activity of muscadine could be beneficial to health.

In summary, the muscadine grape pomace extract was thoroughly characterized regarding its total phenolic content, total flavonoid content, and chemical profiles of phenolics and anthocynidines. These bioactive compounds have shown strong antioxidative activities in terms of the *in vitro* the DPPH free radical scavenging assay, reducing ferric assay, and chelating Fe^{2+} assay. By evaluating the polyphenolics and antioxidant capacity of the muscadine extract, it will help better understanding the potential health benefits of the muscadine products. In addition, the identi-

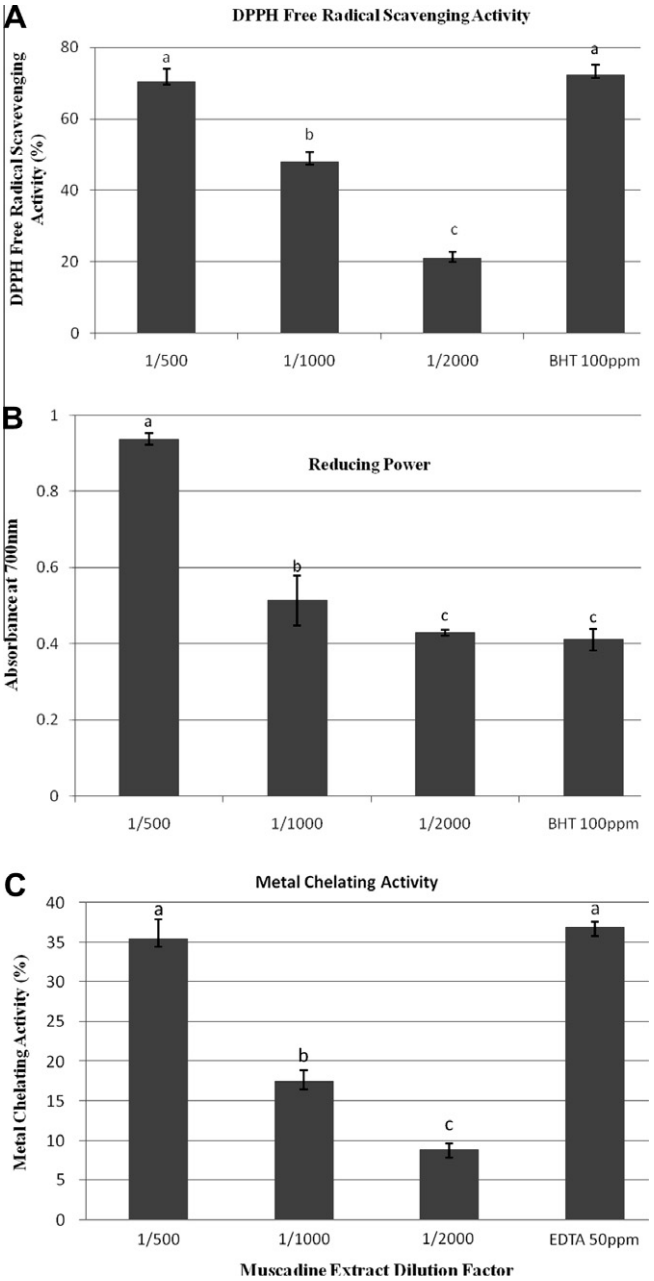

Fig. 4. DPPH free radical scavenging activity of muscadine extract (A); Reducing power of muscadine extract (B); Metal chelating of muscadine extract (C). Error bars represent the standard deviation of triplicate experiments, and different letters represent significant difference at $P < 0.05$.

fication of individual chemicals in the muscadine pomace extract has provided us the base to choose the biomarkers (i.e., flavonoids and anthocynidins bioactive compounds) for quality control. The developed methods can be used for routine quality control of the muscadine products for manufacturing efficiency and consistency.

Acknowledgments

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References

Amerine, M. A., & Ough, C. S. (1980). Phenolic compounds. In W. N. York (Ed.), *Methods of analysis of musts and wines* (pp. 175–199).

AOAC (1990). Moisture in peat. *Association of Official Analytical Chemists* (Vol. 15th ed.). Champaign, IL.

Basha, S., Musingo, M., & Colova, V. (2004). Compositional differences in the phenolics compounds of muscadine and bunch grape wines. *African Journal of Biotechnology, 3*(10), 523–528.

Chung, Y. C., Chang, C. T., Chao, W. W., Lin, C. F., & Chou, S. T. (2002). Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. *Journal Agricultural and Food Chemistry, 50*(8), 2454–2458.

DuPont, M. S., Bennett, R. N., Mellon, F. A., & Williamson, G. (2002). Polyphenols from alcoholic apple cider are absorbed, metabolized and excreted by humans. *The Journal of Nutrition., 132*(2), 172–175.

Garcia-Beneytez, E., Cabello, F., & Revilla, E. (2003). Analysis of grape and wine anthocyanins by HPLC-MS. *Journal Agricultural and Food Chemistry, 51*(19), 5622–5629.

Hong, V., & Wrolstad, R. (1990). Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *Journal Agricultural and Food Chemistry, 38*(3), 709–715.

Hosseinian, F. S., Li, W., & Beta, T. (2008). Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chemistry, 109*, 916–924.

Kim, H. J., Chen, F., Wang, X., & Rajapakse, N. C. (2006). Effect of methyl jasmonate on secondary metabolites of sweet basil (Ocimum basilicum L.). *Journal Agricultural and Food Chemistry, 54*(6), 2327–2332.

Lee, J. H., Johnson, J. V., & Talcott, S. T. (2005). Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. *Journal Agricultural and Food Chemistry, 53*(15), 6003–6010.

Lee, J. H., & Talcott, S. T. (2004). Fruit maturity and juice extraction influences ellagic acid derivatives and other antioxidant polyphenolics in muscadine grapes. *Journal Agricultural and Food Chemistry, 52*(2), 361–366.

Miller, H. E., Rigelhof, F., Marquart, L., Prakash, A., & Kanter, M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of American College of Nutrition, 19*(3), 312s–319s.

Morrissey, P. A., & O'Brien, N. M. (1998). Dietary antioxidants in health and disease. *International Dairy Journal, 8*(5–6), 463–472.

Murias, M., Jäger, W., Handler, N., Erker, T., Horvath, Z., Szekeres, T., et al. (2005). Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure-activity relationship. *Biochemical Pharmacolology, 69*, 903–912.

Nothlings, U., Murphy, S. P., Wilkens, L. R., Henderson, B. E., & Kolonel, L. N. (2007). Flavonols and pancreatic cancer risk: the multiethnic cohort study. *American Journal of Epidemiology, 166*(8), 924–931.

Park, Y. K., Koo, M. H., Ikegaki, M., & Contado, J. L. (1997). Comparison of the flavonoid aglycone contents of Apis mellifera propolis from various regions of Brazil. *Arquivos De Biologia E Tecnologia, 40*(1), 97–106.

Pastrana-Bonilla, E., Akoh, C. C., Sellappan, S., & Krewer, G. (2003). Phenolic content and antioxidant capacity of muscadine grapes. *Journal Agricultural and Food Chemistry, 51*(18), 5497–5503.

Spanos, G. A., & Wrolstad, R. E. (1990a). Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice. *Journal Agricultural and Food Chemistry, 38*(7), 1565–1571.

Spanos, G. A., & Wrolstad, R. E. (1990b). Influence of variety, maturity, processing and storage on the phenolic composition of pear juice. *Journal Agricultural and Food Chemistry, 38*(2), 817–824.

Talcott, S. T., Brenes, C. H., Pires, D. M., & Del Pozo-Insfran, D. (2003). Phytochemical stability and color retention of copigmented and processed muscadine grape juice. *Journal Agricultural and Food Chemistry, 51*(4), 957–963.

Talcott, S. T., & Lee, J. H. (2002). Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. *Journal Agricultural and Food Chemistry, 50*(11), 3186–3192.

USDA (2008). Food and Agricultural Organization of United Nations, Economic and Social Department, The Statistical Division.

Wang, X., Beckham, T. H., Morris, J. C., Chen, F., & Gangemi, J. D. (2008). Bioactivities of gossypol, 6-methoxygossypol, and 6,6'-dimethoxygossypol. *Journal Agricultural and Food Chemistry, 56*(12), 4393–4398.

Yi, W., Fischer, J., & Akoh, C. C. (2005). Study of anticancer activities of muscadine grape phenolics in vitro. *Journal Agricultural and Food Chemistry, 53*(22), 8804–8812.

Yilmaz, Y., & Toledo, R. T. (2004). Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *Journal Agricultural and Food Chemistry, 52*(2), 255–260.

Zhang, H., Wang, X., Chen, F., Androulakis, X. M., & Wargovich, M. J. (2007). Anticancer activity of limonoid from Khaya senegalensis. *Phytotherapy Research, 21*(8), 731–734.

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Phenolic Content and Antioxidant Capacity of Muscadine Grapes

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Fruits of 10 cultivars of muscadine grapes (five bronze skin and five purple skin) grown in southern Georgia were separated into skin, seed, and pulp. Each fruit part and the leaves from the corresponding varieties were extracted for HPLC analysis of major phenolics. Total phenolics were determined colorimetrically using Folin−Ciocalteu reagent. Total anthocyanins were determined according to a pH-differential method, using a UV−visible spectrophotometer. Antioxidant capacity was determined by the Trolox equivalent antioxidant capacity (TEAC) assay. Gallic acid, (+)-catechin, and epicatechin were the major phenolics in seeds, with average values of 6.9, 558.4, and 1299.4 mg/100 g of fresh weight (FW), respectively. In the skins, ellagic acid, myricetin, quercetin, kaempferol, and *trans*-resveratrol were the major phenolics, with respective average values of 16.5, 8.4, 1.8, 0.6, and 0.1 mg/100 g of FW. Contrary to previous results, ellagic acid and not resveratrol was the major phenolic in muscadine grapes. The HPLC solvent system used coupled with fluorescence detection allowed separation of ellagic acid from resveratrol and detection of resveratrol. Reported here for the first time are the phenolic content and antioxidant capacity of muscadine leaves. Major phenolics in muscadine leaves were myricetin, ellagic acid, kaempferol, quercetin, and gallic acid, with average concentrations of 157.6, 66.7, 8.9, 9.8, and 8.6, respectively. Average total phenolics were 2178.8, 374.6, 23.8, and 351.6 mg/g gallic acid equivalent in seed, skin, pulp, and leaves, respectively. Total anthocyanin contents were 2.1 and 132.1 mg/100 g of FW in the skins of bronze and purple grapes, respectively, and 4.3 and 4.6 mg/100 g of FW in seeds and pulps, in that order. Antioxidant capacity values were, on average, 2.4, 12.8, 281.3, and 236.1 μM TEAC/g of FW for pulps, skins, seeds, and leaves, respectively.

KEYWORDS: ***Vitis rotundifolia*; muscadine grapes; phenolics; anthocyanins; antioxidant capacity**

INTRODUCTION

Muscadine grapes (*Vitis rotundifolia* Michx.) are indigenous to the southeastern United States. They are vigorous vines that may grow up to 100 ft in the wild. They differ botanically from other grapes and are placed in a separate subgenus, *Muscadinia.* Muscadine fruits are round, 1−1.5 in. in diameter with a thick, tough skin and may have up to five seeds. The Georgia Agricultural Experiment Station and the U.S. Department of Agriculture have introduced a number of improved varieties that currently are standard cultivars (*1*). Plants contain a large variety of phytonutrients, many having antioxidant properties. Antioxidant compounds include vitamins, phenols, carotenoids, and flavonoids. Among the last group, flavones, isoflavones, flavonones, flavonols, anthocyanins, and catechins are the most important and exhibit substantial antioxidant activity (*2*, *3*). These antioxidants may prevent the incidence of cardiovascular disease (*4*). Phenolics are secondary plant metabolites found in the majority of fruits, vegetables, and teas (*5*). Even though plants are the basis of all traditional medicinal therapy (*6*), the positive effect of antioxidants found in fruits and vegetables was demonstrated by Ames et al. (*7*) and Hertog et al. (*8*−*11*). In the recent years, many studies have demonstrated that free radicals are the leading cause of degenerative diseases such as several forms of cancer, cardiovascular disease, and neurological diseases (*12*). Plant antioxidants work as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, and enzyme inhibitors (*13*). Many of their protective biological effects are derived from their antioxidant functions (*14*). There is interest in knowing the phenolic content of fruits in order to increase their potential use as nutraceuticals or functional foods.

There are few research papers on the phenolic content of muscadine grapes. Ector et al. (*15*), Meepagala et al. (*16*), Goldy

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et al. (*17*), and Talcott and Lee (*18*) represent some of the reports on the phenolic content of muscadine grapes. Studies from our laboratory represent one of the few attempts to measure the polyphenolic content of muscadines and their antioxidant capacity. The objective of this study is to determine the major phenolic compounds found in grapes and leaves, their total polyphenolic content, and the antioxidant capacity of the muscadine fruits and leaves.

MATERIALS AND METHODS

Chemicals. Pure standards of (+)-catechins (95% purity), (−)-epicatechin (90% purity), gallic acid (90% purity), ellagic acid (95% purity), myricetin (85% purity), quercetin (98% purity), kaempferol (90% purity), and *trans*-resveratrol (95% purity) were purchased from Fluka (Milwaukee, WI) and Sigma (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Fluka. Acetonitrile, methanol, and water (HPLC grade) were purchased from Fisher Scientific (Norcross, GA).

Samples. Fruits and leaves from 10 muscadine grape cultivars, namely, five bronze (Carlos, Early Fry, Fry, Summit, and Late Fry) and five purple (Paulk, Cowart, Supreme, Ison, and Noble), grown in southern Georgia were provided by Jacob Paulk (Paulk Vineyards, Wray, GA) and used for this study. Fruits and leaves were randomly collected at the time of optimum harvest maturity, as determined by the grower. Five kilograms of fruits of each cultivar was collected. Homogeneous fruit samples (500 g) were separated into skins, seeds, and pulps in order to determine the percentage of each fruit part with respect to the whole fruit. Each fruit part was packaged, labeled, and stored at −20 °C in the dark until further analysis. Working samples of each fruit part were extracted in triplicate and analyzed as described below. The percentage of each fruit part was used for the calculation of major phenolics, total phenolics, total anthocyanins, antioxidant capacity, and dry matter in whole fruits.

Major Phenolics. Samples of 1 g of skins, 0.5 g of seeds, 0.5 g of leaves, or 2 g of pulps were mashed, using a mortar and pestle, to a very fine paste and diluted with 80% methanol in 6 N HCl. The samples were vortexed for 1 min and then placed in a water bath shaker set at 60 °C and 200 rpm for 2 h for acid hydrolysis of flavonoid glycosides to aglycons. Finally, the samples were vortexed for 1 min to ensure total extraction. The extracted samples were filtered through a 0.2 μm syringe nylon filter and injected into a Hewlett-Packard (Avondale, PA) HP 1090 HPLC system with diode array and fluorescence detectors. The mobile phases were, solvent A, methanol/acetic acid/water (10:2:88, v/v/v); solvent B, acetonitrile; and, solvent C, water. A linear gradient suitable for phenolic separation was used as follows: at 0 min, 100% solvent A; at 5 min, 90% solvent A and 10% solvent B; and at 25 min, 30% solvent A and 70% solvent B, with 5 min postrun with 100% solvent C. The flow rate was 1 mL/min. The Beckman Ultrasphere C18 ODS 4.6 × 250 mm column was used with the column temperature set at 40 °C. The volume of sample injected was 20 μL. All extractions and analyses were performed in the dark to protect the phenolic compounds from degradation.

Total Phenolics. Mashed samples of fruit parts and leaves were extracted in 2% HCl in methanol for 24 h in the dark and at room temperature. The extracts were diluted with the same solvent used for extraction, to a suitable concentration for analysis. Total phenolics were measured according to the Folin−Ciocalteu reagent method (*19*). Two hundred microliters of sample extract was introduced in a test tube, 1.0 mL of Folin−Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) were added, and the contents were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured in a Shimadzu 300 UV−vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample, using a standard curve generated with 100, 200, 300, and 400 mg/L of gallic acid.

Total Anthocyanins. Grape parts (skin, seed, or pulp) were extracted in 2% HCl in methanol for 24 h, following the method described by Revilla et al. (*20*) as the one that gave the highest extraction of anthocyanins in grapes. Total anthocyanin analysis was performed following the method described by Giusti and Wrolstad (*21*) and following their direction for buffer preparation. Skin and seed extracts were diluted to an appropriate concentration with potassium chloride buffer, pH 1, until the absorbance of the sample was within the linear range of the Shimadzu 300 UV−vis spectrophotometer (0−1.2). The spectrophotometer was zeroed with distilled water. Two dilutions of each sample were prepared, one with potassium chloride buffer, pH 1, and the other with sodium acetate buffer, pH 4.5, and the dilutions were allowed to equilibrate for 15 min. The absorbance was measured at 520 and at 700 nm (to correct for haze) against a blank cell filled with distilled water, following the pH differential method described by Giusti and Wrolstad (*21*).

Antioxidant Capacity. The antioxidant capacity was determined as Trolox equivalent antioxidant capacity (TEAC), following a slight modification to the method described by Re et al. (*22*). Trolox, a vitamin E analogue, was used as an antioxidant standard. ABTS was dissolved in water to a concentration of 7 mM and allowed to react with a 2.45 mM potassium persulfate solution for 16 h in the dark. This reaction will form ABTS radical cations ($ABTS^{\bullet+}$). The $ABTS^{\bullet+}$ solution was diluted in ethanol to an absorbance of 0.70 (± 0.02) at 734 nm; 1.980 mL of diluted $ABTS^{\bullet+}$ solution was drawn with an automatic pipet and placed into a quartz cuvette, and after exactly 1 min, 20 μL of antioxidant compound or Trolox standard was added and mixed. The absorbance reading was recorded for up to an additional 6 min. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data. The ratio between the area under the curve for the reaction of the specific antioxidant and that for Trolox gave the relative antioxidant capacity. For calibration, Trolox standards of 0, 300, 600, 900, 1200, and 1500 μM were prepared to obtain final concentrations in the cuvette of 0, 3, 6, 9, 12, and 15 μM, respectively (antioxidant sample corresponds to 1% of the total solution in the cuvette). Data from the spectrophotometer were saved as Excel files (Microsoft Corp.). The area under the curve was calculated using the software TableCurve 2D V5.0 (SPSS Inc., Chicago, IL).

Dry Weight Determination. Sample dry weight was determined following the guidelines of the official AOAC method 967.03 (*23*). For each cultivar, 500 g of fruits was separated into pulps, seeds, and skins. Each fruit part fraction was weighed, and the composition of the fruit (as percentage of its fruit parts) was determined. Approximately 10 g of each fruit part was placed into an aluminum pan (in triplicate) and dried for 16 h in an oven set at 105 °C. After the drying time, the pans were removed from the oven, allowed to cool in a desiccator, and weighed, and the dry weight was determined as grams of dry matter per gram of sample.

Statistics. The statistical analysis was carried out using the Microsoft Excel software package (Microsoft Corp.).

RESULTS AND DISCUSSION

The major phenolics in muscadine grape skins were identified by their retention times and characteristic spectra. Quantification was made by calibration curves of external standards built for each of the analyzed compounds, (+)-catechin, (−)-epicatechin, gallic acid, ellagic acid, *trans*-resveratrol, and the aglycons of myricetin, quercetin, and kaempferol. Ellagic acid, resveratrol, and the aglycons of myricetin, quercetin, and kaempferol were found in muscadine skins, whereas (+)-catechin, (−)-epicatechin, and gallic acid were found in the seeds. To check the performance of the extraction method, some skin and seed samples were spiked with selected phenolics and analyzed for recovery. Recoveries of ellagic acid and resveratrol were 95.8 and 98.7%, respectively, when skin samples were spiked with known amounts of the compounds. The recovery of gallic acid was 83.5% when seed samples were spiked. **Table 1** shows the individual phenolic compounds in whole muscadine grapes. **Table 2** shows the major phenolics identified and quantified in the skins and seeds of the 10 selected cultivars of muscadine

Table 1. Phenolics in Muscadine Grapes (Milligrams per 100 g of Fresh Whole Fruit)[a]

cultivar	ellagic acid	myricetin	quercetin	kaempferol	resveratrol	(–)-epicatechin	(+)-catechin	gallic acid	% skin	% seeds	% pulp
bronze											
Carlos	6.4	6.3	0.4	0.1	0.1	71.8	86.1	0.6	32.3	6.0	61.6
Early Fry	7.0	5.8	0.6	0.1	0.1	32.4	19.0	0.1	35.7	2.0	62.3
Fry	5.7	1.8	1.1	0.4	0.1	33.1	6.4	0.1	43.3	1.8	54.9
Summit	5.4	4.2	1.8	1.4	0.1	6.9	5.4	0.1	45.8	1.5	52.7
Late Fry	9.9	5.6	0.4	0.1	nd[b]	74.0	19.9	0.4	46.7	3.9	49.4
av	6.8	4.7	0.9	0.4	0.1	43.6	27.4	0.3	40.8	1.9	56.2
SD[c]	1.8	1.8	0.6	0.6	0.0	28.7	33.5	0.2	6.4	1.8	5.6
purple											
Paulk	6.0	0.7	0.7	0.2	nd	30.4	5.8	0.2	40.7	1.8	57.5
Cowart	7.4	2.2	0.3	0.1	0.1	60.3	17.7	0.3	34.2	5.1	60.7
Supreme	3.0	1.0	1.4	0.1	0.1	17.1	5.1	nd	47.8	1.1	51.1
Ison	8.7	2.8	0.5	0.2	0.1	30.9	19.2	0.3	39.1	3.5	57.3
Noble	6.8	2.2	0.2	0.2	0.1	66.6	30.7	1.1	46.2	9.2	44.6
av	6.4	1.8	0.6	0.2	0.1	41.1	15.7	0.4	41.6	4.1	54.2
SD	2.1	0.9	0.5	0.1	0.0	21.3	10.6	0.4	5.5	3.2	6.4

[a] Values are the average of triplicates. [b] Not detected. [c] Standard deviation.

Table 2. Phenolics in Muscadine Grape Parts (Milligrams per 100 g of Fresh Weight of Fruit Part)[a]

	skins					seeds		
cultivar	ellagic acid	myricetin	quercetin	kaempferol	resveratrol	(–)-epicatechin	(+)-catechin	gallic acid
Carlos	19.7 ± 2.0	19.6 ± 2.3	1.3 ± 0.2	0.4 ± 0.0	0.2 ± 0.0	1189.2 ± 51.9	1424.7 ± 30.3	9.4 ± 0.4
Early Fry	19.7 ± 1.0	16.4 ± 1.1	1.6 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	1603.0 ± 88.7	940.5 ± 80.1	3.3 ± 0.2
Fry	13.1 ± 0.8	4.1 ± 0.0	2.5 ± 0.1	0.8 ± 0.0	0.2 ± 0.1	1850.7 ± 553.3	355.6 ± 54.2	4.5 ± 0.3
Summit	11.7 ± 1.1	9.2 ± 0.7	3.8 ± 0.2	3.0 ± 0.2	0.2 ± 0.1	450.1 ± 81.2	348.5 ± 67.1	5.0 ± 0.4
Late Fry	21.1 ± 2.0	12.1 ± 1.0	0.9 ± 0.1	0.2 ± 0.0	nd	1897.6 ± 598.0	511.3 ± 38.4	9.5 ± 1.0
Paulk	14.7 ± 1.1	1.8 ± 0.1	1.7 ± 0.5	0.4 ± 0.0	nd	1672.2 ± 478.8	319.6 ± 131.4	9.9 ± 1.2
Cowart	21.6 ± 1.7	6.4 ± 0.6	0.9 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	1180.6 ± 27.7	347.2 ± 27.0	5.0 ± 0.1
Supreme	6.2 ± 0.3	2.0 ± 0.4	3.0 ± 0.6	0.2 ± 0.0	0.2 ± 0.0	1553.8 ±179.8	460.6 ± 129.4	2.2 ± 0.5
Ison	22.2 ± 1.6	7.1 ± 0.6	1.4 ± 0.2	0.6 ± 0.1	0.2 ± 0.0	872.6 ± 4.9	542.3 ± 79.6	8.8 ± 1.1
Noble	14.6 ± 0.2	4.8 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	724.2 ± 60.5	333.5 ± 32.6	11.5 ± 1.1

[a] Values are the average and standard deviation of triplicates; nd, not detected.

grapes. The phenolic content in the whole fruit was calculated on the basis of the results of individual phenolic compounds in the skins and seeds shown in **Table 2** and the percentage of each fruit part. Ellagic acid was the most abundant phenolic compound in muscadine grape skins with concentrations from 6.2 to 22.2 mg/100 g of fresh weight (FW); myricetin had concentrations from 1.8 to 19.6 mg/100 g of FW; quercetin varied from 0.5 to 3.8 mg/100 g of FW; kaempferol was found at concentrations from 0.2 to 3.0 mg/100 g of FW; and *trans*-resveratrol had the lowest concentrations of the detected phenolics, ranging from not detected in two varieties to 0.2 mg/100 g of FW (**Tables 1** and **2**). Our result for resveratrol differed from previous results (*15*) indicating high concentrations. These researchers apparently were not able to separate ellagic acid from resveratrol with UV detection alone. Another possible reason for the discrepancy in resveratrol concentration could be due to the varietal and agroecological differences between our grapes and those of the previous researchers. However, we were able to separate the two compounds with our solvent system, identify and quantify them with UV detection, and confirm the resveratrol identity and amount with fluorescence detection. The fluorescence detector was set at wavelengths of 330 and 374 nm (*24*, *25*), for excitation and emission, respectively. **Figure 1** shows the spectra of ellagic acid and *trans*-resveratrol for standards and a sample of the skin of the muscadine grape cv. Carlos. The HPLC chromatogram of selected standards for muscadine skin analysis, showing diode array at different wavelengths, and fluorescence detection are

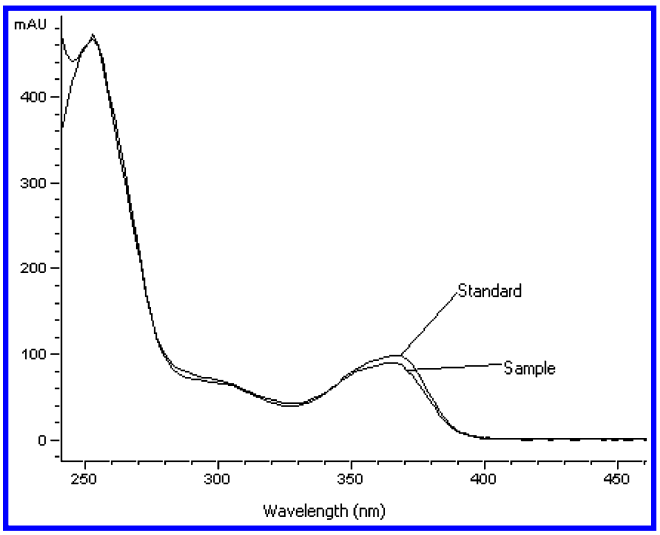

Figure 1. Diode array spectra of ellagic acid for standards and a sample of the skin of the cv. Carlos.

in **Figure 2**. **Figures 3** and **4** show the HPLC chromatograms of selected standards and a sample of the skin of the muscadine grape cv. Carlos, respectively. The major phenolics detected in muscadine seeds (**Tables 1** and **2**) were (−)-epicatechin, (+)-catechin, and gallic acid. (−)-Epicatechin concentration ranged from 450.1 to 1897.6 mg/100 g of FW, (+)-catechin had concentrations between 319.6 and 1424.7 mg/100 g of FW, and

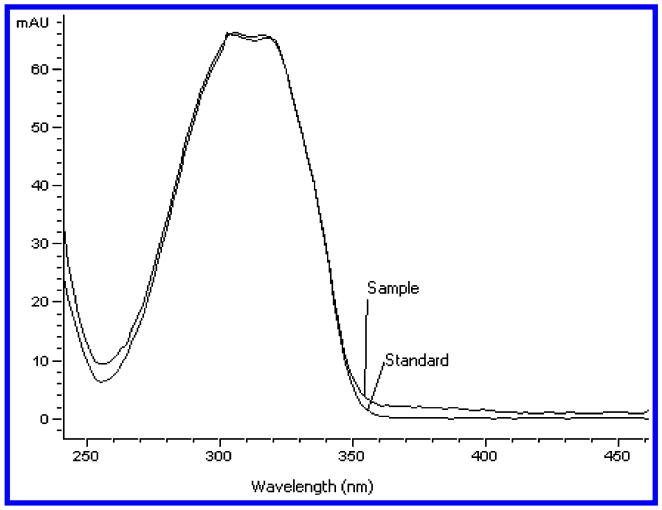

Figure 2. Diode array spectra of *trans*-resveratrol for standards and a sample of the skin of the cv. Carlos.

gallic acid varied from 2.2 to 11.5 mg/100 g of FW. Major phenolics in muscadine leaves (**Table 3**) were myricetin, ellagic acid, kaempferol, quercetin, and gallic acid. Myricetin varied from 107.7 to 216.4 mg/100 g of FW, which on the average is 50 times its concentration in the whole muscadine grape and 18 times the concentration in the skin. Ellagic acid ranged between 44.8 and 80.0 mg/100 g of FW, which is 10 times the concentration of ellagic acid in the fruit and 4 times the concentration in the skin. Kaempferol was found in concentrations between 5.7 and 11.5 mg/100 g of FW, corresponding to 32 times the concentration of the phenolic compound in the fruit and 14 times the concentration in the skin. The flavonol quercetin ranged from 6.3 to 21.6 mg/100 g of FW, corresponding to 13 times the concentration in the whole fruit and 6 times the concentration in the skin. Gallic acid varied from 6.1 to 18.7 mg/100 g of FW, which is, on average, 29 times the concentration of gallic acid in fruit and about the same concentration as in the seed. No phenolics (from the ones that we analyzed) were detected in the grape pulps. Varietal differences between bronze-skinned and purple-skinned grapes were found only in the case of myricetin and total anthocyanin contents. Myricetin content was higher in the bronze-skinned grapes, and total anthocyanin content was higher in purple-skinned grapes. Leaves of both varieties had similar polyphenolic content, and no significant difference was found.

The total phenolics in muscadine grape parts were, on average, 5 times more concentrated in the seed than in the skin and 80 times more than in the pulp (**Table 4**). This result may be due to the high concentration of catechins in the seed and the very low presence of major phenolics in pulp. The relatively high value for total phenolics in skins in comparison to the sum of individual phenolics found in them indicates that some other phenolics may be present in the skins but not identified in this study. The whole muscadine fruits had, on average, 50% less total phenolics than the leaves (**Table 5**), even though the skins and seeds had high contents of phenolics. However, the high relative weight of the pulp to the skins and seeds and their very low content of phenolics contributed to a low phenolics value in the whole fruit. Nevertheless, it is important to note that in muscadine grape processing, as in juice, wine, or jelly production, skins and seeds are discarded as waste. Therefore, the nutraceutical industry may use the muscadine seeds and skins

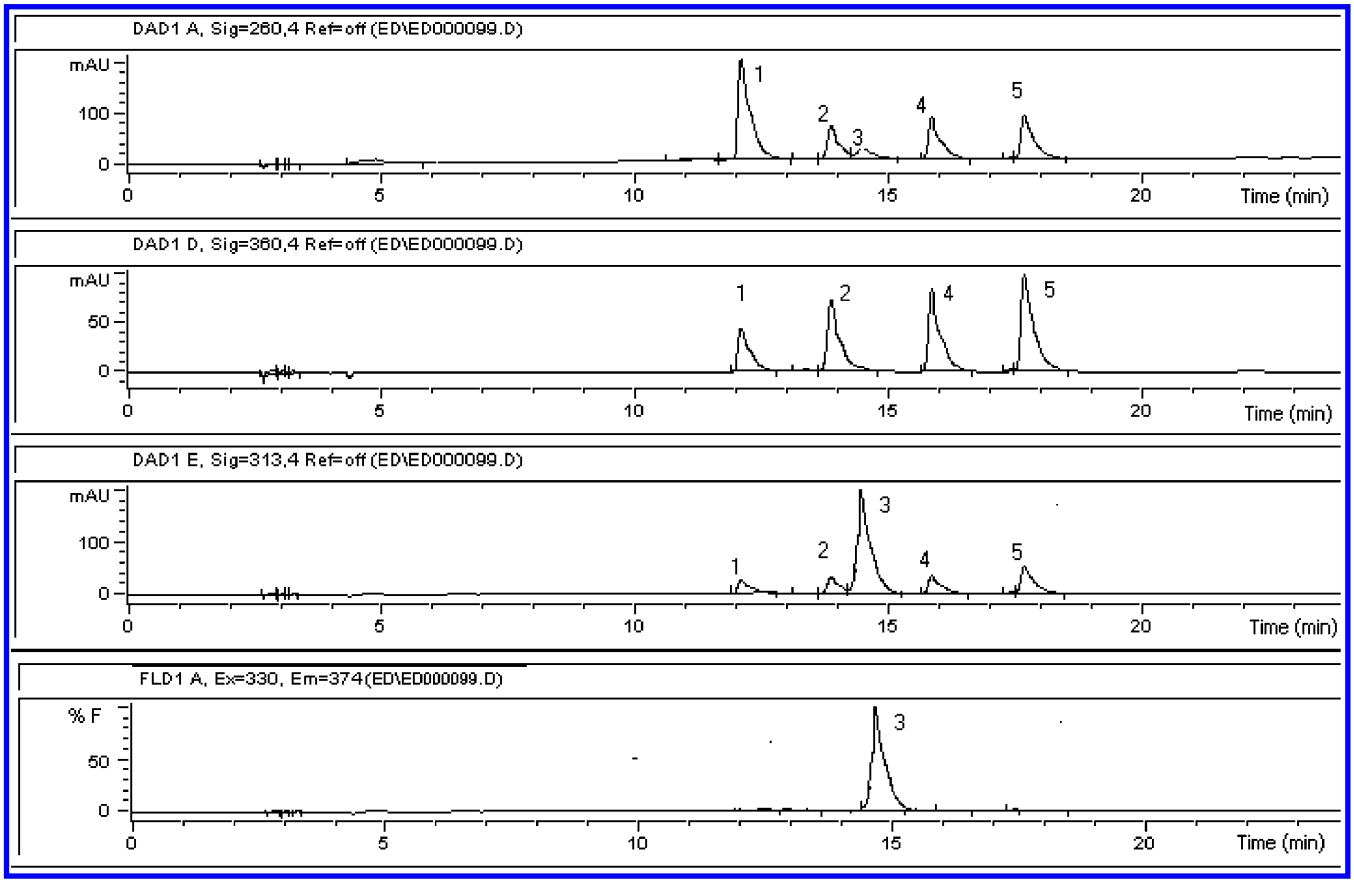

Figure 3. HPLC chromatograms of selected standards for muscadine skin analysis at 260 nm (DAD1 A), 360 nm (DAD1 D), and 313 nm (DAD1 E) and fluorescence detection at Ex = 330 nm and Em = 374 nm (FLD1 A) of ellagic acid (1), myricetin (2), *trans*-resveratrol (3), quercetin (4), and kaempferol (5).

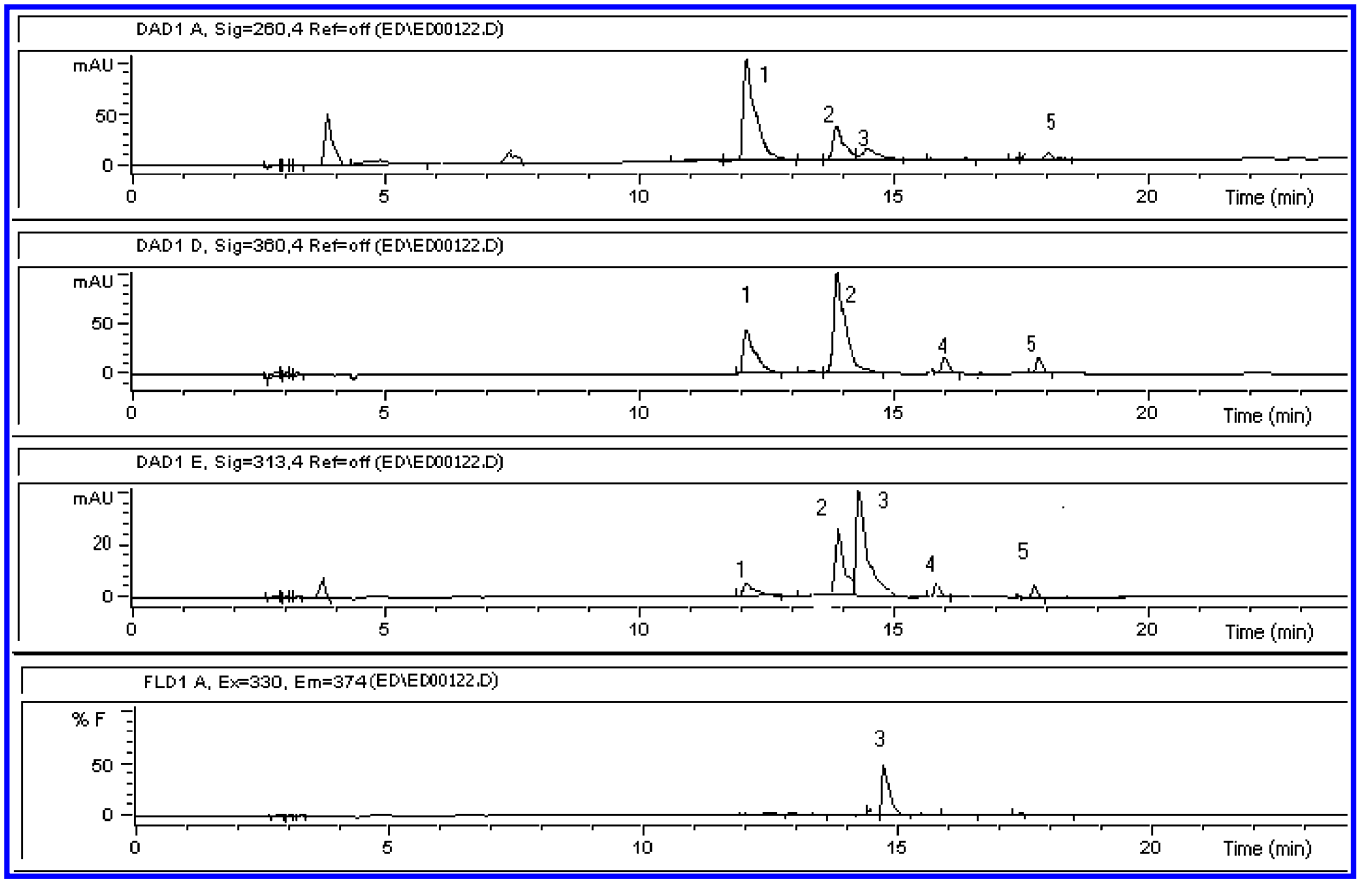

Figure 4. HPLC chromatograms of the skin of grapes of the cv. Carlos at 260 nm (DAD1 A), 360 nm (DAD1 D), and 313 nm (DAD1 E) and fluorescence detection at Ex = 330 nm and Em = 374 nm (FLD1 A) of ellagic acid (1), myricetin (2), *trans*-resveratrol (3), quercetin (4), and kaempferol (5).

Table 3. Major Phenolics in Muscadine Leaves (Milligrams per 100 g of Fresh Weight)[a]

cultivar	ellagic acid	kaempferol	myricetin	quercetin	gallic acid
bronze					
Carlos	80.0 ± 5.9	8.7 ± 1.4	145.8 ± 4.2	8.0 ± 1.0	7.6 ± 0.1
Early Fry	79.0 ± 4.6	10.6 ± 1.3	216.4 ± 4.9	21.6 ± 1.5	9.3 ± 0.2
Fry	76.7 ± 4.4	8.8 ± 0.4	140.9 ± 3.3	7.9 ± 0.3	6.1 ± 0.2
Summit	55.6 ± 2.7	5.7 ± 0.2	107.7 ± 3.5	6.3 ± 0.3	6.9 ± 0.3
Late Fry	66.1 ± 3.2	10.2 ± 1.1	162.1 ± 4.6	8.1 ± 0.5	8.3 ± 0.3
av ± SD[b]	71.5 ± 10.5	8.8 ± 1.9	154.6 ± 39.8	10.4 ± 6.3	7.6 ± 1.2
purple					
Paulk	65.5 ± 5.0	10.0 ± 1.0	166.2 ± 5.3	8.4 ± 0.7	6.4 ± 0.9
Cowart	74.7 ± 3.7	7.9 ± 0.3	157.1 ± 4.8	11.8 ± 1.0	7.8 ± 0.7
Supreme	59.1 ± 2.8	7.2 ± 0.3	133.8 ± 5.7	9.9 ± 0.9	7.7 ± 0.6
Ison	65.1± 4.0	11.5 ± 1.0	178.5 ± 6.3	8.0 ± 0.7	7.1 ± 0.7
Noble	44.8 ± 2.8	8.6 ± 0.7	167.3 ± 4.9	7.9 ± 0.6	18.7 ± 2.8
av ± SD	61.8 ± 11.0	9.0 ± 1.7	160.6 ± 17.8	9.2 ± 1.7	9.5 ± 5.2

[a] Values are the average and standard deviation of triplicates. [b] Standard deviation.

as potential sources of phenolics, and for the farmers or processors they may provide an additional source of income. Leaves are allowed to remain in the field after the fruits have been harvested and finally will fall to the ground during the autumn. They could also be collected and used for the extraction of polyphenolics or processed for use as functional foods.

The analysis of total anthocyanins showed that bronze-skinned muscadine grapes had very low anthocyanin content in skins and seeds and no anthocyanins in pulps. The seeds of this group of fruits had higher relative anthocyanin content. For purple-skinned muscadine grapes, the skins showed higher anthocyanin content, ranging from 65.5 to 177.0 mg/100 g of FW expressed as cyanidin 3-glucoside. This corresponds to 65 times more anthocyanins than in the skins of bronze grapes. The total anthocyanin content in the seeds of purple grapes was 1.3 times higher than in bronze grape seeds, and the pulps of purple grapes had, on average, 2 mg/100 g of FW. This could be due to some migration of the pigments from the skin to the pulp or some tinting from ruptured skin cells during the process of separation of the fruit into its parts. **Tables 4** and **5** show the results for total anthocyanins. No total anthocyanin analysis was performed on the leaves after they were collected at the time of harvest for the fruits, and at that physiological stage it is possible to assume that muscadine leaf cells do not have such pigments.

Tables 4 and **5** also show the antioxidant capacity data. The total average values were 12.8, 281.3, 2.4, 15.3, and 236.1 μM Trolox equiv/g of FW in skins, seeds, pulps, whole grapes, and leaves, respectively. This means that seeds had 22, 116, and 18 times more antioxidant capacity than skins, pulps, and the whole grapes, respectively. Additionally, seeds had, on average, 20% more antioxidant capacity than leaves. The seeds had ~6 times more total phenolics than the leaves, and the ratio of antioxidant capacity/total phenolics was ~6 times higher for the leaves than the seeds. It is presumable that major phenolics in leaves have higher antioxidant capacity than the ones found in seeds or that other antioxidant compounds different from phenolics may be present in higher concentrations in the leaves than in the seeds. A comparison of our results with those reported by Wang and Lin (*13*) indicated that the antioxidant capacity of muscadine leaves was at least twice the value for the leaves of some berry plants, such as blackberry, raspberry, or strawberry.

Table 6 shows the results for dry weight determination of muscadine grapes and the corresponding dry weight of the whole

Table 4. Total Phenolics, Total Anthocyanins, and Antioxidant Capacity of Muscadine Grape Parts

	total phenolics (GAE mg/100 g of FW)			total anthocyanins (mg/100 g of FW as cyanidin 3-glucoside)			TEAC[a] (μM/g of FW)		
cultivar	seed	skin	pulp	skin	seed	pulp	skin	seed	pulp
bronze									
Carlos	1920.3	545.6	25.1	2.6	1.2	nd[b]	14.9	204.6	3.4
Early Fry	2367.2	303.0	21.3	2.5	8.7	nd	13.9	277.8	2.0
Fry	2356.3	332.2	23.8	0.8	4.6	nd	11.1	234.2	2.9
Summit	3258.7	541.0	22.3	2.8	3.1	nd	12.4	245.4	3.0
Late Fry	1986.0	348.9	24.0	2.0	3.7	nd	13.4	218.9	2.4
av ± SD[c]	2377.7 ± 533.7	414.1 ± 119.1	23.3 ± 1.5	2.1 ± 0.8	4.3 ± 2.8	nd	13.1 ± 2.5	236.2 ± 37.9	2.7 ± 0.5
purple									
Paulk	1649.3	363.6	30.0	177.0	4.1	4.7	12.1	307.9	2.2
Cowart	2303.0	261.6	11.6	107.8	4.6	1.1	12.4	325.5	2.7
Supreme	1535.5	329.9	20.1	135.5	7.5	0.7	12.2	478.6	1.6
Ison	1726.2	365.0	26.0	174.5	4.6	1.9	13.3	284.9	2.1
Noble	2685.3	355.1	33.4	65.5	2.2	2.2	12.4	234.7	2.1
av ± SD	1979.9 ± 493.2	335.0 ± 43.4	24.2 ± 8.6	132.1 ± 47.0	4.6 ± 1.9	2.1 ± 1.6	12.5 ± 0.5	326.3 ± 91.7	2.1 ± 0.4

[a] Trolox equivalent antioxidant capacity; values are the average of triplicates. [b] Not detected. [c] Standard deviation.

Table 5. Total Phenolics (TPH), Total Anthocyanins (TAC), and Trolox Equivalent Antioxidant Capacity (TEAC) of Muscadine Grapes and Leaves[a]

	TPH (GAE mg/100 g of FW)		TAC (mg/100 g of FW)	TEAC (μM/g of FW)	
cultivar	whole fruit	leaves	whole fruit	whole fruit	leaves
bronze					
Carlos	307.9	350.1	0.9	18.2	229.8
Early Fry	169.1	437.0	1.1	11.2	251.0
Fry	199.0	340.4	0.4	9.8	239.8
Summit	309.7	282.1	1.3	10.2	222.0
Late Fry	252.3	355.0	1.1	15.4	235.0
av ± SD[b]	247.6 ± 63.3	352.9 ± 55.3	1.0 ± 0.3	13.0 ± 3.7	235.5 ± 10.9
purple					
Paulk	195.2	356.5	74.8	11.2	247.8
Cowart	214.2	359.3	37.8	21.7	164.0
Supreme	184.7	317.7	65.2	11.5	304.0
Ison	218.9	370.4	69.5	15.9	283.0
Noble	425.7	347.3	31.5	27.8	184.8
av ± SD	247.7 ± 100.5	350.2 ± 20.0	55.8 ± 19.7	17.6 ± 7.1	236.7 ± 60.8

[a] Values are the average of triplicates. [b] Standard deviation.

Table 6. Dry Matter of Muscadine Grape Fruits and Fruit Parts (Grams per Gram of Fresh Weight)[a]

cultivar	skin	seed	pulp	whole fruit
Carlos	0.179	0.532	0.137	0.174
Early Fry	0.159	0.562	0.149	0.161
Fry	0.139	0.523	0.144	0.148
Summit	0.165	0.571	0.166	0.180
Late Fry	0.161	0.516	0.152	0.162
Paulk	0.163	0.578	0.146	0.159
Cowart	0.149	0.531	0.121	0.139
Supreme	0.169	0.514	0.137	0.186
Ison	0.182	0.559	0.157	0.184
Noble	0.135	0.596	0.122	0.151

[a] Values are the average of triplicates.

fruit calculated by taking into account the percentage of each fruit part in the whole fruit. This was provided as additional information to simplify the comparison of our results to those of other fruits for which the results were reported on a dry weight basis.

Most phenolics in grapes were located in the seeds and skins. Muscadine pulps have a very low content of phenolics. The main phenolics in muscadines were ellagic acid, kaempferol, myricetin, and quercetin. Seeds of muscadine grapes had higher antioxidant capacity compared to the other fruit parts, which may be due to their high concentrations of catechin and epicatechin. Because the leaves have 15 times more antioxidant capacity than the fruits and the seeds and skins have high polyphenolic contents, they have the potential to be processed for use as functional foods or nutraceuticals. This paper contains the first report of myricetin in muscadine grapes and of phenolics in muscadine leaves.

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LITERATURE CITED

(1) California Rare Fruit Growers, Inc. http://www.crfg.org/pubs/ff/muscadinegrape.html, 1999.

(2) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity and prooxidant behavior of flavonoids: Structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(3) Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 304−309.
(4) Schramm, D. D.; German, J. B. Potential effects of flavonoids on the etiology of vascular disease. *J. Nutr. Biochem.* **1998**, *9*, 560−566.
(5) Amakura, Y.; Umino, Y.; Tsuji, S.; Tonogai, Y. Influence of jam processing on the radical scaveging activity and phenolic content in berries. *J. Agric. Food Chem.* **2000**, *48*, 6292−6297.
(6) Zheng, W.; Wang, S. Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165−5170.
(7) Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.
(8) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem.* **1992**, *40*, 2379−2383.
(9) Hertog, M. G. L.; Hollman, P. C. H.; Putte, B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Food Chem.* **1993**, *41*, 1242−1246.
(10) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen eldery study. *Lancet* **1993**, *342*, 1007−1011.
(11) Hertog, M. G. L.; Kromhout, D.; Aravanis, C. Flavonoid intake and long-term risk of coronary-heart-disease and cancer in the 7 countries study. *Arch. Intern. Med.* **1995**, *155*, 381−386.
(12) Prior, R. L.; Cao, G.; Martin, A.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolics and anthocyanin content, maturity, and variety of *Vaccinum* species. *J. Agric. Food Chem.* **1998**, *46*, 2686−2693.
(13) Wang, S. Y.; Lin, H.-S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140−146.
(14) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.
(15) Ector, B. J.; Magee, C. P.; Coign, M. J. Resveratrol concentration in muscadine berries, juice, pomace, purees, seeds, and wines. *Am. J. Enol. Vitic.* **1996**, *47*, 57−62.
(16) Meepagala, K. M.; Magee, J. B.; Wedge, D. E. Phenolic constituents from muscadine grape (*Vitis rotundifolia*) cultivar polyanna seeds. *Abstr. Pap. Am. Chem. Soc.* **2002**, *No. 223*, 039-AGFD.
(17) Goldy, R. G.; Ballinger, W. E.; Maness, E. P.; Shallow, W. H. Anthocyanin content of fruit, stem, tendril, leaf, and leaf petioles in muscadine grape. *J. Am. Soc. Hortic. Sci.* **1987**, *112*, 882−882.
(18) Talcott, T.; Lee, J.-H. Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. *J. Agric. Food Chem.* **2002**, *50*, 3186−3192.
(19) Singleton, V. L.; Rossi, J. A., Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(20) Revilla, E.; Ryan, J.-M.; Martin-Ortega, G. Comparison of several procedures used for the extraction of anthocyanins from red grapes. *J. Agric. Food Chem.* **1998**, *46*, 4592−4597.
(21) Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by UV−visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Acree, T. E., An, H., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Sporns, P., Eds.; Wiley: New York, 2001; pp F1.2.1−F1.2.13.
(22) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying and improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(23) Association of Official Analytical Chemists. Moisture in peat. *Official Methods of Analysis*, 15th ed.; AOAC: Champaign, IL, 1990; Method 967.03.
(24) Viñas, P.; López-Erroz, C.; Marín-Hernández, J. C.; Hernández-Córdoba, M. Determination of phenols in wines by liquid chromatography with photodiode array and fluorescence detection. *J. Chromatogr. A* **2000**, *871*, 85−93.
(25) Pezet, R.; Pont, V.; Cuenat, P. Method to determine resveratrol and pterostilbene in grape berries and wines using high-performance liquid chromatography and highly sensitive fluorimetric detection. *J. Chromatogr. A* **1994**, *196*, 522−525.

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