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TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 38
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

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Bogotá, D.C.,

Abogado(a)/Señor(a)
ALICIA LLOREDA RICAURTE

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 Evento: 378
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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/EP2010/050929				
Fecha de Presentación Internacional	27/01/2010				

1. Documentos estudiados

Descripción:	Radicado N°11-087831-00000-00000	13/Julio/2011
Reivindicaciones:	Radicado N°11-087831-00000-00000	13/Julio/2011
	Radicado N°11-087831-00002-00000	29/Septiembre/2011
Prioridad:	EP 09151383.8	27/Enero/2009

2. Análisis de la Prioridad

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

3. 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.

Para el caso de la presente solicitud, al verificar la tasa pagada, todo el ultimo capitulo reivindicatorio presentado se tendrá en cuenta para realizar el examen de patentabilidad.

Título de la solicitud: “Composición que comprende ácido caftárico y/o derivados del mismo”

El problema planteado en esta solicitud es la necesidad de proporcionar una composición con ácido caféico y ácido tartárico sin que pierdan su actividad, que sean estables y fácil de producir.

Para resolver este problema técnico la solicitud en estudio proporciona una composición con unos determinados componentes, debidamente definida (radicado N°11-087831-00000-00000, Pág. 6).

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 23 L 1/30.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 9 – 10 no es patentable, de acuerdo con el Art 14, puesto que las reivindicaciones mencionan el uso de la composición.

5. De la Solicitud de Patente

Título

El título no corresponde con el objeto de la solicitud porque la expresión “derivados del mismo” no está debidamente sustentado y además no se menciona el otro componente principal de la composición, por lo cual se sugiere al Solicitante hacer la corrección necesaria.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no se encuentra debidamente soportadas en la descripción puesto que en las realizaciones preferidas hace referencia a la preparación y actividad de la composición cuyos componentes están mencionados en la tabla 1, lo cual no evidencia la actividad de todos los componentes sino que se hace una generalización de manera especulativa.

Se sugiere restringir la solicitud a una razonable cantidad de acuerdo al tipo de formulaciones probadas, es decir, que se encuentran debidamente soportados en el capítulo descriptivo. Teniendo en cuenta la descripción en los ejemplos se encuentra:

Tabla 1. Ejemplos de los componentes de la composición

Componente	Soporte de los ejemplos presentados
Bacterias lácticas	<i>Lactobacillus jhonsoni</i> La1, <i>Bifidobacterium longum</i> BB536, <i>Bifidobacterium lactis</i> BB12
Enzimas	Clorogenato esterasa, Feruloil esterasa
Ácido caftarico	

El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción.

El capítulo reivindicatorio no se encuentra debidamente sustentada en la descripción puesto que en las realizaciones preferidas no se menciona un alimento ya sea producto alimenticio, complemento alimenticio o bebida que contenga ácido caftárico, sino que los ensayos se hacen es con el ácido caftárico directamente, lo cual no asegura que al

hacerlo en un alimento de los mencionados se observen los mismos resultado puesto que son matrices más complejas que pueden presentar interferencias ya sea con inhibir las bacterias o las enzimas. Por lo tanto no se puede hacer alusión a un alimento en el capítulo reivindicatorio sino solo con el ácido caftárico directamente.

La reivindicación 1 no es clara puesto que no se sabe cuántos son los componentes de la composición, puesto que en las realizaciones preferidas aparentemente son alrededor de 3 componentes, pero este debe quedar claramente mencionado en las reivindicaciones.

La reivindicación 1 hace referencia a una composición pero no se mencionan los componentes y las proporciones de los mismos, por lo tanto se sugiere mencionar cuales son los componentes y sus proporciones. Puesto que hacen parte de las características esenciales de la invención.

En las reivindicaciones 1 y 2 no son claras al mencionarse los términos y expresiones “ingrediente que contiene caftárico”, “bacteria de ácido láctico”, “fuente de proteína”, “fuente de carbohidrato” y “fuente de lípido” se están haciendo alusión a unos términos muy amplios, puesto que para una persona de nivel medio versada en la materia, estos evocan su función y no se está indicando la denominación común de los compuestos y las bacterias. Se sugiere definir a qué tipo, tipos, estructura o familia de bacterias lácticas, fuente de proteína, fuente de carbohidrato y fuente de lípido se está haciendo referencia, pues no se puede definir sólo por su función. De acuerdo a lo sustentado en la descripción.

Las reivindicaciones 1 y 6 no son claras porque mencionan la expresión “capaz de hidrolizar ácido caftárico y/o derivados del mismo para generar ácido tartárico y/o caféico son bacterias probióticas...” que corresponden a un resultado a alcanzar, que no definen a la composición porque la invención no queda definida por el resultado a alcanzar, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Retirar dicha expresión.

Las reivindicaciones 1 – 3, 6 no son clara porque la expresión “o” es disyuntiva, lo que quiere decir que es alternativo emplear los componentes que preceden dicha expresión. Se sugiere al solicitante establecer cuál de las dos componentes se pretende reivindicar y retirar dicha expresión.

Las reivindicaciones 11 y 12 no son claras puesto que aunque en su preámbulo mencionan un equipo se está definiendo por la composición con sus determinados componentes, lo cual no hace parte de las características esenciales de un equipo. Además el equipo no se encuentra debidamente sustentado en la descripción puesto que en las realizaciones preferidas no se hace alusión a este. Por lo tanto se deben retirar dichas reivindicaciones.

6. Unidad de invención (Art 25 D 486)

El concepto común nuevo e inventivo que plantea la solicitud correspondería a una composición que comprende ácido caftárico y una bacteria de ácido láctico. Pero esta composición es ampliamente conocida en los documentos D1 a D4, por lo tanto no es posible reconocer un único concepto común nuevo e inventivo para la solicitud. Además se plantean composiciones y un equipo para preparar tal composición que resuelven el problema técnico de interés. De tal manera se evidencian 2 grupos inventivos debido a las diferencias de las reivindicaciones. Los Grupos Inventivos están definidos por las siguientes características **esenciales**:

1º grupo inventivo: Composición comprende ácido caftárico y una bacteria de ácido láctico (Reiv. 1-8)

2º grupo inventivo: Equipo para preparar producto alimenticio (Reiv. 14-15)

Se sugiere presentar las solicitudes divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención eliminando de ella las otras invenciones.

7. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad, Enero 27 de 2009 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	EP1911358	"New composition for improving skin quality and a process for preparing the same"	16/Abril/2008
D2	WO2004000038	"Composition and method to produce and use fermented hydrolyzed products of metabolism"	31/Diciembre/2003
D3	US4671962	"Chicory base drink and method of preparing same"	09/Junio/1987
D4	US2006240077	"Methodologies for improving the quality of meat, health status of animals and impact on environment"	26/Octubre/2006

- El documento D1 ^[1] divulga una composición que comprende Polifenoles de extracto de vino rojo, donde estos son ácidos hidroxicinámicos, junto con bacterias ácido láctico, tales como *Lactobacillus casei*, *Bifidobacterium longum*, entre otras. (Pág. 2, Párr. [0012]; Pág. 3, Párr. [0033]; Pág. 4, Párrs. [0035], [0037] y [0045])
- El documento D2 ^[2] divulga una composición que comprende uvas, Berry roja y negra, espinaca entre otros, junto con bacterias lactobacillus que incluyen *Lactobacillus johnsonii* y Bacterias Bifidobacterium que incluye *Bifidobacterium longum*, *Bifidobacterium lactis*. (Pág. 6, Párr. [0019]; Pág. 10, Párr. [0040] y [0043]; Págs. 11 y 12, Párr. [0050]).
- El documento D3 ^[3] divulga una bebida de chicory que comprende raíces de chicory que se fermenta con *Lactobacillus casei alactosus*, *Lactobacillus cellobiosus*, *Streptococcus lactis*, entre otros. (Col. 3, líneas 52 – 62; Col. 5, líneas 44 – 63)
- El documento D4 ^[4] divulga un producto de chicory que comprende un extracto de raíces de chicory que puede ser fermentado con bacterias, *Lactobacillus casei alactosus*, *Lactobacillus cellobiosus*, *Streptococcus lactis*, entre otros (Págs. 7 y 8, Párr. [0083]; Pág. 8, Párrs. [0091], [0093], [0094]; Pág. 10, Párrs. [0117] – [0119]).

8. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La **reivindicación 1** consiste en una composición cuyos componentes se encuentran definidos (Rad. N° 11-087831-000002-00000. Pág. 27)

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

1⁰URL:http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20080416&CC=EP&NR=1911358A1&KC=A1

2⁰URL:http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20031231&CC=WO&NR=2004000038A1&KC=A1

3⁰URL:http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19870609&CC=US&NR=4671962A&KC=A

4⁰URL:http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20061026&CC=US&NR=2006240077A1&KC=A1

Características esenciales	D1	D2	D3	D4
Composición que comprende:	Composición que comprende:	Composición que comprende:	Bebida de chicory que comprende:	Producto de chicory que comprende:
Ácido caftárico	Polifenoles de extracto de vino rojo, donde estos son ácidos hidroxicinámicos	Uvas, Berry roja y negra, espinaca entre otros	Raíces de chicory	Extracto de raíces de chicory
Bacteria ácido láctico	Bacteria ácido láctico	Bacterias lactobacillus que incluyen <i>Lactobacillus johnsonii</i> y Bacterias Bifidobacterium que incluye <i>Bifidobacterium longum</i> , <i>Bifidobacterium lactis</i>	<i>Lactobacillus casei alactosus</i> , <i>Lactobacillus cellobiosus</i> , <i>Streptococcus lactis</i> , entre otros	<i>Lactobacillus casei alactosus</i> , <i>Lactobacillus cellobiosus</i> , <i>Streptococcus lactis</i> , entre otros

Los documentos D1 a D4 son considerados el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

D1 divulga una composición que comprende Polifenoles de extracto de vino rojo, donde estos son ácidos hidroxicinámicos, junto con bacterias ácido láctico, tales como *Lactobacillus casei*, *Bifidobacterium longum*, entre otras. Además contiene proteínas de la leche, ácidos grasos poliinsaturados, entre otros (Pág. 2, Párr. [0012]; Pág. 3, Párr. [0033]; Pág. 4, Párrs. [0035], [0037] y [0045])

D2 divulga una composición que comprende uvas, Berry roja y negra, espinaca entre otros, junto con bacterias lactobacillus que incluyen *Lactobacillus johnsonii* y Bacterias Bifidobacterium que incluye *Bifidobacterium longum*, *Bifidobacterium lactis*. Además de proteínas, vitaminas, aminoácidos, entre otros (Pág. 6, Párr. [0019]; Pág. 10, Párr. [0040] y [0043]; Págs. 11 y 12, Párr. [0050]).

D3 divulga una bebida de chicory que comprende raíces de chicory que se fermenta con *Lactobacillus casei alactosus*, *Lactobacillus cellobiosus*, *Streptococcus lactis*, entre otros. (Col. 3, líneas 52 – 62; Col. 5, líneas 44 – 63).

D4 divulga un producto de chicory que comprende un extracto de raíces de chicory que puede ser fermentado con bacterias, *Lactobacillus casei alactosus*, *Lactobacillus cellobiosus*, *Streptococcus lactis*, entre otros, además puede contener metabolitos secundarios tales como terpenos, derivados de ácido caféico, entre otros (Págs. 7 y 8, Párr. [0083]; Pág. 8, Párrs. [0091], [0093], [0094]; Pág. 10, Párrs. [0117] – [0119]).

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D1 consiste en que no se especifica la presencia del ácido caftárico, sino que en D1 se menciona los polifenoles de la familia de los ácidos hidroxicinámicos.

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D2 consiste en que no se especifica la presencia del ácido caftárico, sino que en D2 se menciona son los frutos uvas y berrys.

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D3 consiste en que no se especifica la presencia del ácido caftárico, sino que en D3 se menciona son las raíces de chicory.

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D4 consiste en que no se especifica la presencia del ácido caftárico, sino que en D4 se menciona son el extracto de raíces de chicory.

El efecto que lograría las diferencias antes mencionada en la composición de la reivindicación 1, en cuanto a mantener la actividad y estabilidad de sus componentes, no puede ser determinado toda vez que no existe alguna evidencia que indique que dichas diferencias en especificar la presencia del ácido caftárico, otorgan a la composición ahora reivindicada una ventaja técnica sobre las composiciones ya conocidas en la anterioridad D1 o D2 o D3 o D4.

Por lo tanto, el problema técnico objetivo a resolver consiste en plantear una alternativa a las composiciones conocidas según D1 o D2 o D3 o D4.

Sin embargo es conocido en el estado de la técnica que el ácido caftárico está presente en la planta chicory y en las uvas, principalmente en el vino blanco, lechuga, espinaca lo cual es divulgado en diferentes estudios que evidencian la presencia del ácido caftárico, que al ser hidrolizado proporciona ácido tartárico y caféico. Además que el ácido caftárico pertenece a lo polifenoles, especialmente hace parte de los llamados ácidos hidroxicinámicos y presenta una actividad antioxidante.^{5,6,7,8}

Teniendo en cuenta lo expuesto anteriormente, la personas de nivel medio versada en la materia derivara de manera obvia la composición descrita en la reivindicación 1 de la presente invención a partir de las enseñanzas de la composición del documento D1 o D2 o D3 o D4 y de incluir el hecho que ya es conocido que el ácido caftárico está presente en la planta chicory y en las uvas, principalmente en el vino, por lo tanto las diferencias se limitan en especificar un compuesto ampliamente conocido en ciertos alimentos, sin que ese hecho confiera que no solamente reduzca sino elimine la parestesia, incluyendo las razas de perros pequeños, y que controle las plagas.. Por lo tanto al combinar D1 o D2 o D3 o D4 con lo conocido en el estado de la técnica en los artículos científicos.

Puesto que las reivindicaciones dependientes 2 – 4, 6 – 8 y 11 – 12 no mencionan características inventivas adicionales, tampoco puede ser considerada inventiva.

Conclusión:

Las reivindicaciones 1 – 4, 6 – 8 y 11 – 12, cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

9. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	EP1911358	1 – 4, 6 – 8 y 11 – 12	Nivel inventivo
D2	WO2004000038	1 – 4, 6 – 8 y 11 – 12	Nivel inventivo
D3	US4671962	1 – 4, 6 – 8 y 11 – 12	Nivel inventivo
D4	US2006240077	1 – 4, 6 – 8 y 11 – 12	Nivel inventivo

5 Gonthier M., et Al. Microbial metabolism of caffeic acid and its esters chlorogenic and caftaric acids by human faecal microbiota in vitro. Biomedicine & Pharmacotherapy 60, pp 536–540, (2006).

6 Clifford M. Review: Chlorogenic acids and other cinnamates-nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture. Vol 79, pp 362.372 (1999)

7 Singleton, J. et Al. Caftaric acid in grapes and conversion to a reaction product during processing. Department of Vi ti culture and Enology, University of California, Da vis, California, USA. Vitis 23, pp 113-120 (1984)

8 Vernon L., et Al. Caftaric and coutaric acids in fruit of *Vitis*. Phytochemistry, Vol. 25, No. 9. pp. 2127-2133, 1986.

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 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.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-08-27

Elaboró: Jhonattan Sanchez Sanchez
Revisó: Gloria Jacqueline Alfonso Roza

Microbial metabolism of caffeic acid and its esters chlorogenic and caftaric acids by human faecal microbiota in vitro

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Abstract

Caffeic acid and its esters, chlorogenic and caftaric acids, are major dietary polyphenols present in various foods and beverages. Although caffeic acid is easily absorbed in the small intestine, its esterification with quinic acid, as in chlorogenic acid, decreases its gut absorption and increases the quantities reaching the colon and its microbiota. The microbial conversion of caftaric acid, the tartaric acid ester of caffeic acid, has not been studied earlier. In this work we compared the direct action of a human faecal microbiota on the metabolism of caffeic, chlorogenic and caftaric acids in an in vitro fermentation model. All substrates disappeared quickly and none of the free acids (caffeic, quinic or tartaric acids) were detected after 2 hours of incubation. Two major microbial metabolites were identified by HPLC–ESI–MS–MS as 3-hydroxyphenylpropionic (3-HPP) and benzoic acids (BA). Maximal levels of 3-HPP were reached after 2 h of fermentation and accounted for 9–24% of the dose of caffeic acid and its esters. BA was formed steadily throughout the incubation, accounting for 4–5% of the initial dose of the substrates after 24 h of incubation. The similarities in the metabolic patterns observed for caffeic, chlorogenic and caftaric acids suggest that esterification does not influence the metabolism of caffeic acid by the gut microbiota.

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Keywords: Polyphenols; Caffeic acid; Chlorogenic acid; Caftaric acid; Faecal microbiota; In vitro metabolism

1. Introduction

Hydroxycinnamic acids constitute a major class of polyphenols widely distributed in fruits, vegetables and some beverages. Caffeic acid is one of the most abundant hydroxycinnamic acids in the human diet. It can be found either free or esterified with either quinic or tartaric acids (Fig. 1). Among the quinic acid conjugates, 5-*O*-caffeoylquinic acid, commonly referred to as chlorogenic acid, is the best known and is abundant in coffee, blueberry, apple and cider. In grape, spinach, lettuce and wine, the main derivative of caffeic acid is 2-*O*-caffeoyltartaric acid, also called caftaric acid [1].

In common with several other dietary polyphenols, caffeic acid and its esters chlorogenic and caftaric acids have free hydroxyl groups and can act as antioxidants in vitro [2–4]. However, our knowledge on the bioavailability of these caffeic acid derivatives is still too limited to fully assess their impact on biological functions in humans. Several studies have established that caffeic acid is efficiently absorbed through the small intestine and is found in the body either intact or in glucuronidated, sulphated and *O*-methylated forms (ferulic and isoferulic acids), increasing the total antioxidant status of plasma [5–7]. Different experiments on rats and with human volunteers have shown that chlorogenic acid is also absorbed, but to a lesser extent than caffeic acid [6,8–11]. Intestinal esterase and glucosidase activities, both of tissues and of the microbiota, play an important role in the uptake of esterified acids and glycosylated polyphenols [12–16]. Polyphenols are extensively degraded to

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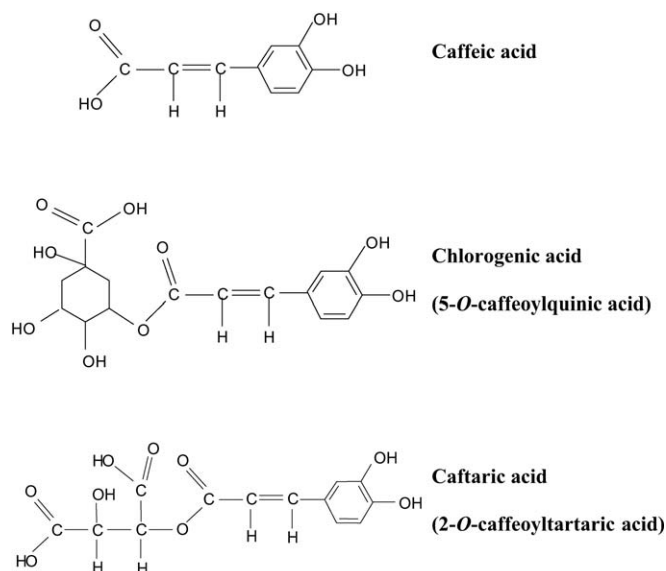


Fig. 1. Chemical structures of caffeic, chlorogenic and caftaric acids.

various derivatives of phenylacetic, phenylpropionic and benzoic acids (BA), which are excreted in urine both in man and in rats [9,10,17–20]. The importance of the microbial metabolites from major dietary polyphenols, including caffeic acid, has also been illustrated in vitro [21–25]. Gut microbiota may play a crucial role in the bioavailability of poorly absorbed polyphenols, and may contribute to their health effects.

The aim of this study was to compare the metabolism of caffeic, chlorogenic and caftaric acids by a human faecal microbiota in an in vitro fermentation model. The effect of caffeic acid esterification with either quinic or tartaric acids on its microbial degradation was evaluated for the first time.

2. Materials and methods

2.1. Materials

Caffeic and chlorogenic acids were purchased from Sigma Chemical (St. Louis, MO, USA). *Trans*-caftaric acid was purified from Grenache grapes and characterised by reverse-phase HPLC–UV as described previously [26]. Quinic, tartaric, 3-hydroxybenzoic, 4-hydroxybenzoic, protocatechuic, vanillic, hippuric, syringic, 3-hydroxyphenylacetic, 3,4-dihydroxyphenylacetic, 3-coumaric, 4-coumaric, ferulic and isoferulic acids were purchased from Sigma Chemical. 3-Hydroxyphenylpropionic (3-HPP) and 3,4-dihydroxyphenylpropionic acids were obtained from Apin Chemicals Limited (Abingdon, UK). 3-Hydroxyhippuric acid was kindly provided by P.C.H. Hollman (RIKILT, Wageningen University, The Netherlands) and 4-hydroxyhippuric acid by R. R. Scheline (University of Bergen, Norway).

2.2. In vitro faecal fermentation experiment

Fermentation of caffeic acid, chlorogenic acid and caftaric acid were performed according to the method previously

described [22] with the following modifications: Faecal slurry was prepared by dilution to 5% (w/v) with a buffer solution (pH 5.5) under strictly anaerobic conditions using faeces from four healthy donors. Part of the slurry was autoclaved (121 °C for 20 min) to produce heat-inactivated flora. Fermentation bottles with caffeic acid, chlorogenic acid or caftaric acid (1 μmol) were inoculated with either active or inactive human faecal slurry (10 ml) and incubated with stirring for 0, 2, 4, 6, 8 or 24 h in anaerobic conditions at 37 °C. Faecal slurry without added phenolic compounds was used as a control. After incubation, the conversion was stopped by rapid freezing and samples were freeze-dried. All experiments were conducted in triplicate. The results were calculated per 10 ml of faecal suspension on a molar basis and expressed as mean $\pm$ S.E.M.

2.3. Extraction and analysis of caffeic, chlorogenic and caftaric acids and their metabolites

Freeze-dried faecal samples were suspended in 5 ml of sodium acetate buffer (0.1 mol/l, pH 5) and supplemented with syringic acid as an internal standard (5 $\mu\text{mol/l}$). After acidification to pH 4.9 with 0.5 ml acetic acid (0.58 mol/l), samples were incubated at 37 °C for 45 min in the presence of *Helix pomatia* extract containing 1100 U β -glucuronidase and 42 U sulphatase (Sigma Chemical). Twenty millilitre of methanol containing 200 mmol/l HCl were added and the mixture was centrifuged at 4500 rpm for 10 min at 4 °C. The resulting supernatant was filtered (PTFE membrane, 0.45 μm , Millipore, Bedford, USA) and a 40 μl -aliquot of the filtrate directly injected into the HPLC–ESI–MS–MS system.

HPLC–ESI–MS–MS analyses were performed in a Hewlett–Packard HPLC system equipped with MS–MS detection (API 2000, Applied Biosystem, Canada), as previously described [18]. Detection and quantification of compounds was achieved with Multiple Reaction Monitoring mode, based on the respective m/z values of parent and product ions corresponding to the 22 compounds selected for analysis (Table 1). For quantification, blank faecal samples were supplemented with six concentrations of a mixture of standards (0, 0.5, 1, 2.5, 5, 10 $\mu\text{mol/l}$), extracted and analysed as described above. Calibration curves were constructed by plotting peak areas against the corresponding concentrations. Recoveries of parent phenolic compounds and their identified metabolites were determined after addition of the different standards to a blank faecal sample. These recoveries were 78, 70, and 95% for caffeic, chlorogenic and caftaric acids, respectively. For the identified metabolites, 3-HPP acid and BA, recoveries were 82% and 87%, respectively. These values were used to correct the concentrations determined in the incubated samples.

3. Results

Caffeic acid, chlorogenic acid and caftaric acid (1 μmol) were incubated in the presence of a human faecal microbiota and the metabolites were analysed by HPLC–ESI–MS–MS. The initial substrate dose was 1 μmol , from which $68.9 \pm 7.9\%$,

Table 1

Phenolic acid metabolites looked for in the faecal fermentation samples by tandem mass spectrometry. Negative ionisation was used.

Compounds	Molecular weight	Parent ion (m/z)	Product ion (m/z)
<i>Parent compounds</i>			
Caffeic acid	180	179	135
Chlorogenic acid	354	353	191
Caftaric acid	312	311	149
<i>Metabolites</i>			
Quinic acid	192	191	85
Tartaric acid	150	149	73
3,4-Dihydroxyphenylpropionic acid	182	181	59
3-HPP	166	165	121
3-Coumaric acid	164	163	119
4-Coumaric acid	164	163	119
Ferulic acid	194	193	134
Isoferulic acid	194	193	134
3,4-Dihydroxyphenylacetic acid	168	167	123
3-Hydroxyphenylacetic acid	152	151	107
Protocatechuic acid	170	169	125
Vanillic acid	168	167	123
3-Hydroxyhippuric acid	195	194	150
4-Hydroxyhippuric acid	195	194	100
Hippuric acid	179	178	134
3-Hydroxybenzoic acid	138	137	93
4-Hydroxybenzoic acid	138	137	93
BA	122	121	77
<i>Internal standard</i>			
Syringic acid	198	197	123

53.5 ± 6.3%, and 96.0 ± 2.0% for caffeic, chlorogenic and caftaric acids, respectively, was recovered at the initial time point (0 h) from active faecal microbiota. Incubation with the active faecal microbiota resulted in rapid disappearance of caffeic acid and its esters (Fig. 2A–C). Neither free caffeic acid nor quinic or tartaric acids could be detected in the presence of active microbiota (results not shown). The recoveries of the parent compounds in the presence of heat-inactivated microbiota varied at the initial time point (0 h): 66.9 ± 5.4%, 46.8 ± 6.2% and 107 ± 8.5% for caffeic, chlorogenic and caftaric acids, respectively. The concentrations of the parent compounds remained constant during the incubation, indicating that they were not modified in the presence of inactivated microbiota.

Two compounds, subsequently identified as 3-HPP and BA, were detected among a series of known polyphenol metabolites (Table 1), together with the parent phenolic acids. The appearance of 3-HPP was evident for all the substrate compounds. The production reached its maximum after 2 hours of incubation (Fig. 3). The extent of 3-HPP formation varied according to the esterification of the substrate. The maxima for 3-HPP formation were 0.13 ± 0.02 µmol, 0.09 ± 0.03 µmol, and 0.24 ± 0.02 µmol from the initial dose (1 µmol) of caffeic, chlorogenic and caftaric acids, respectively. The concentration of 3-HPP returned to the baseline after 4 h of incubation, indicating further degradation of this bacterial metabolite. 3-HPP was absent from the faecal background.

BA was formed at a slower rate from caffeic acid and its esters than 3-HPP (Fig. 4). The extent of BA formation reached maxima for caffeic acid and caftaric acid (0.040 ± 0.003 µmol

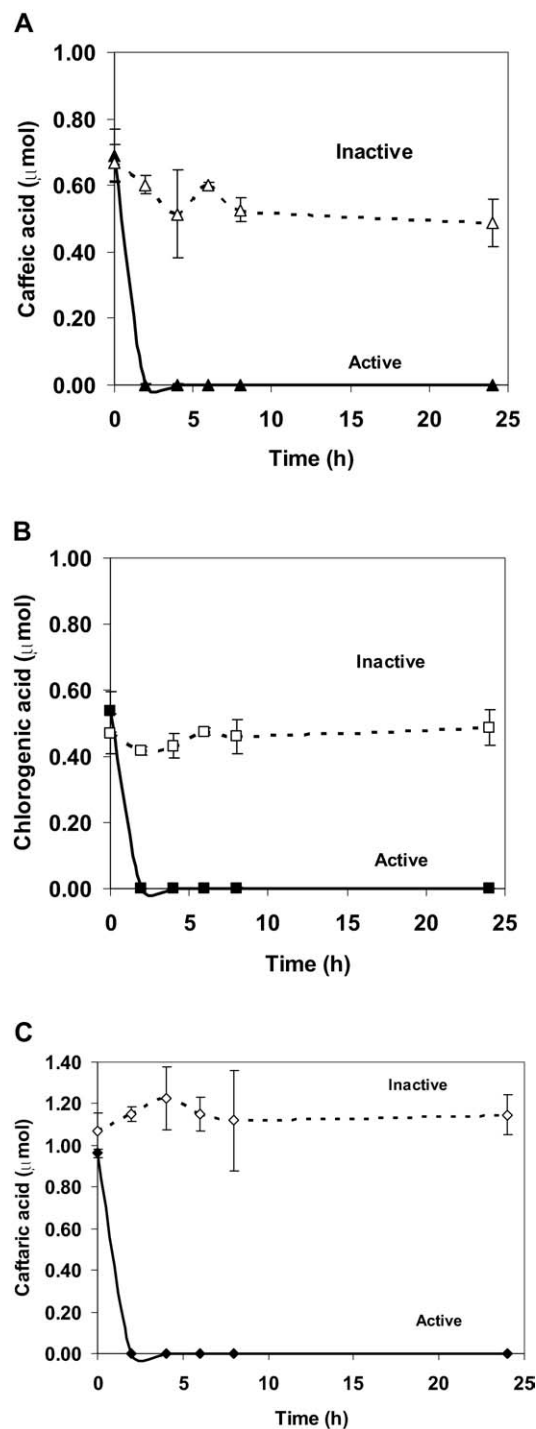


Fig. 2. Concentration of caffeic acid (▲, △), chlorogenic acid (■, □) or caftaric acid (◆, ◇) during incubation with active (closed symbols) or with heat-inactivated (open symbols) human faecal microbiota. Values are expressed as mean ± S.E.M. (N = 3).

and 0.040 ± 0.002 µmol, respectively) in 8 h and for chlorogenic acid (0.046 ± 0.004 µmol) in 24 h, and only less than 5% (mol/mol) of the initial dose of the substrate was converted to BA. The incubation with heat-inactivated faecal microbiota showed no formation of either of the bacterial metabolites 3-HPP or BA (results not shown).

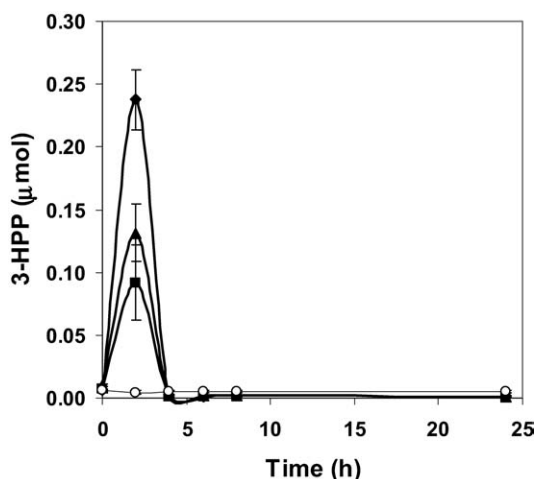


Fig. 3. Formation of 3-HPP during incubation with active human faecal microbiota in the presence of caffeic (▲), chlorogenic (■) and caftaric (◆) acids and in their absence (○). Values are expressed as mean $\pm$ S.E.M. ($N = 3$).

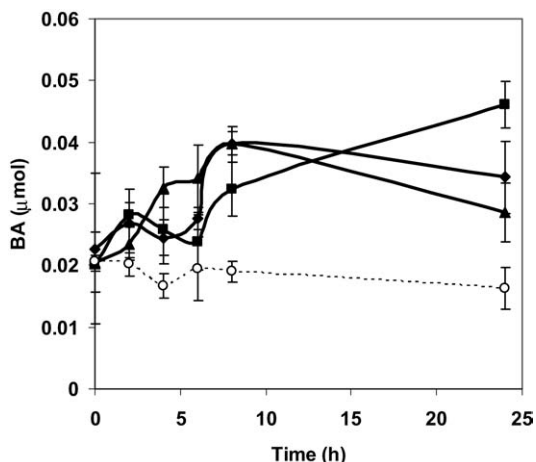


Fig. 4. Formation of BA during incubation with active faecal microbiota in the presence of caffeic (▲), chlorogenic (■) and caftaric (◆) acids and in their absence (○). Values are expressed as mean $\pm$ S.E.M. ($N = 3$).

4. Discussion

In this work, we used an *in vitro* fermentation model with human faecal microbiota to study the microbial metabolism of caffeic acid and its esters, chlorogenic acid and caftaric acid. The substrate compounds disappeared within 2 hours. Direct evidence for hydrolysis of caffeic acid ester by microbial esterases present in the colon has previously been provided by incubation of chlorogenic acid with human faecal samples [13]. Since free quinic, tartaric or caffeic acids were not detected, their rapid metabolism to other molecules was probable.

We identified two microbial metabolites as 3-HPP and BA. 3-HPP was the major microbial degradation product of caffeic acid and its esters. 3-HPP, formed from caffeic acid by reduction of a double bond and dehydroxylation at the C4 position, appeared within two hours and disappeared after four hours, suggesting further conversion for all the three substrates used in this study. Esterification of caffeic acid was not a rate-

limiting factor in the conversion, suggesting that chlorogenic and caftaric acids are easily hydrolysed by the faecal microbiota. Clinical trials showed the presence of several hydroxylated cinnamic and hippuric acids in human urine after chlorogenic acid supplementation [5,9,17]. The present study demonstrates the role of faecal microbiota in the formation of these metabolites in man and the results presented here are in good agreement with the *in vivo* metabolite profile in man [5,9,17].

Small amounts of BA were also formed slowly throughout the whole fermentation from all the caffeic acid derivatives. Evidence for a metabolic pathway leading to the formation of BA from 3-HPP is supported by the established property of intestinal micro-organisms to carry out biological dehydroxylation, particularly that of 3-HPP to 3-phenylpropionic acid. 3-Phenylpropionic acid can itself be further β -oxidised into BA by the colonic microbiota [25,27–32]. Both cinnamic and phenylpropionic acids undergo β -oxidation in the liver to produce BA, which is subsequently conjugated to glycine to form hippuric acid in the liver [5,9,31]. The amount of the formed BA was so low that the differences between samples containing caffeic acid and its esters cannot be considered significant. However, the slow *in vitro* formation of BA in the present study was concomitant with a decrease in 3-HPP concentration, confirming the ability of the human faecal microbiota to metabolise the primary metabolite into BA.

3-HPP was detected as the main metabolite of caffeic acid and its esters and accounted for 9–24% (mol/mol) of the initial dose of the substrates. It is most likely that the primary metabolite, 3-HPP, was rapidly metabolised to 3-phenylpropionic acid, as indicated in by Déprez et al. [21] after formation of 3-HPP from proanthocyanidins. However, 3-phenylpropionic acid was not among the analysed metabolites. The other metabolites could also include gaseous metabolites, which were not analysed in this study. For example carbon dioxide formation has been shown to occur from quercetin in man [33].

In conclusion, the similarities observed in the metabolic pattern obtained for caffeic acid and its two esters clearly establish that caffeic acid esterification with either quinic or tartaric acids does not affect its degradation by the colonic microbiota. To our knowledge, this is the first report on the microbial metabolism of caftaric acid. Whether microbial metabolites have any physiological significance is not clear at present. They might exert local effects on the colon mucosa. As the microbial metabolites, formed in the colon have been found in urine, they are evidently absorbed and might therefore also exert systemic effects. Long residence time in man (up to 35 h) has been demonstrated for microbial metabolites of polyphenols, suggesting long time of effect [34,35]. Antioxidant and anti-platelet aggregation activities of such metabolites have been described [36–39]. Evaluation of their biological significance should be further investigated.

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Review

Chlorogenic acids and other cinnamates – nature, occurrence and dietary burden[†]

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Abstract: This review defines the range of forms in which cinnamates (*p*-coumarates, caffeates, ferulates and sinapates) occur in foods and beverages subdividing them into (i) the classic chlorogenic acids and close allies, (ii) other esters, amides and glycosides, and (iii) transformation products formed during processing. Cinnamate derivatives which would not release cinnamic acid by hydrolysis are excluded. The quantitative data are reviewed concisely and attention is drawn to certain shortcomings, in particular a complete absence of data for certain commodities (breakfast cereals, baked goods, tomato products and nuts) and minimal data for pulses, legumes and processed or cooked foods. In addition, more data are required for the edible portion of modern varieties. By extrapolating from such data as are available the important source(s) (i) of individual cinnamates (regardless of the conjugate type) and (ii) of each major class of conjugate, have been identified as follows:

- (i) *Cinnamates*: caffeic acid: coffee beverage, blueberries, apples, ciders; *p*-coumaric acid: spinach, sugar beet fibre, cereal brans; ferulic acid: coffee beverage, citrus juices, sugar beet fibre, cereal brans; sinapic acid: broccoli, kale, other leafy brassicas, citrus juices.
- (ii) *Conjugates*: caffeoylquinic acids: coffee beverage, blueberries, apples, ciders; *p*-coumaroylquinic acids: sweet cherries; feruloylquinic acids: coffee beverage; tartaric conjugates: spinach, lettuce, grapes and wines; malic conjugates: lettuce, spinach, possibly legumes; rosmarinic acid: culinary herbs, mixed herbs, possibly stuffings; cell wall conjugates: spinach, sugar beet fibre, cereal brans.

It seems likely that the UK population will fall into several categories depending on (i) their consumption of coffee, (ii) their consumption of bran, and (iii) their consumption of citrus. Those who drink several cups of coffee per day augmented by bran and citrus might easily ingest 500–800 mg *cinnamates* (or even 1 g for the greatest coffee ingest consumption) whereas those who eschew all these and take little fresh fruit or vegetables might struggle to consume 25 mg.

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Keywords: review; chlorogenic acid; cynarin; cinnamates; caffeic; ferulic; *p*-coumaric; sinapic; caftaric; coumaric; fertaric; chicoric; phaseolic; avenanthramides; fruits; vegetables; berries; pome fruits; stone fruits; citrus; grapes; legumes; brassicas; herbs; cereals; beers; wines; tea; coffee; cocoa; cider; maté; bran; fibre; human diet; Apiaceae; Asteraceae; Boraginaceae; Chenopodiaceae; Fabaceae; Lamiaceae; Rosaceae; Solanaceae; Vitaceae

INTRODUCTION

Possibly the first report referring to chlorogenic acids was that published in 1837 by Robiquet and Boutron,¹ but the term itself does not seem to have been introduced until 1846 by Payen.^{2,3} In 1932 chlorogenic acid was shown to be a caffeic acid conjugate of quinic acid.⁴

Since then a vast literature has developed and it has become apparent that there are many cinnamoylquinic conjugates, that these are only one of many conjugated forms of the cinnamic acids, and

that such conjugates are extremely widespread in the plant kingdom. It is not easy to summarise this literature and even less easy to put this information into the context of human food. Many of the data available relate to non-food sources although some plants studied may be close botanical relatives of plants consumed and others may be of interest as herbal remedies. Even when foodstuffs have been analysed, data are often qualitative, or for a very limited number of samples and varieties (not necessarily those of current horticultural or agricultural

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importance) and quite often not solely for edible tissue. Only occasionally has consideration been given to maturity or season and rarely to the effects of processing or cooking.

It is not easy to identify unequivocally one component as a particular conjugate among many possible and structurally similar substances and it is now known that some early identifications were in error. For example, the original identification of cinnamoyl-glycerols in pineapple as 1,4-di-*p*CoQA,⁵ the incorrect identification of a caffeoyltartaric acid glucoside in grapes and of 1,5-diCQA in coffee, corrected by Baranowski and Nagel⁶ and Clifford⁷ respectively, and the early confusion over the conjugates in cranberries discussed by Marwan and Nagel.⁸ There may be others as yet unrecognised, especially in view of some apparent inconsistencies in the data available.

THE NATURE OF THE CINNAMATE CONJUGATES

The chlorogenic acids

Classically, chlorogenic acids (CGA) are a family of esters formed between certain *trans*-cinnamic acids and quinic acid (1L-1(OH),3,4/5-tetrahydroxycyclohexane carboxylic acid) which has axial hydroxyls on carbons 1 and 3, and equatorial hydroxyls on carbons 4 and 5. During processing, *trans* isomers may be partially converted to *cis*.

Using the preferred IUPAC numbering,⁹ the commonest individual chlorogenic acid is 5-*O*-caffeoylquinic acid (5-CQA). It is the only one commercially available and is still often called chlorogenic acid or 3-CQA (pre-IUPAC numbering) but the use of both terms should be discouraged. (Please note that in this review all citations will use the IUPAC system and if necessary numbering has been changed for consistency and clarity.)

These classic CGA may be subdivided by the identity, number and position of the acyl residues. The following subgroups are known:

- the relatively widespread mono-esters of caffeic acid, ie caffeoylquinic acids (CQA), *p*-coumaroylquinic acids (*p*CoQA) and feruloylquinic acids (FQA);
- di-esters, tri-esters and the single tetra-ester of caffeic acid, ie diCQA, triCQA and tetraCQA, the latter two groups being characteristic of Asteraceae;^{10–14}
- mixed di-esters of caffeic and ferulic acid, ie caffeoylferuloylquinic acids (CFQA) which are characteristic of robusta coffee (*Coffea canephora*, Rubiaceae)^{15–18} or caffeic and sinapic acid, ie caffeoylsinapoylquinic acids (CSiQA) as found in *Gardenia jasminoides* (Rubiaceae);¹⁹
- mixed esters involving various permutations of between one and three residues of caffeic acid with one or two residues of a dibasic aliphatic acid (eg glutaric, oxalic, succinic) which are characteristic of Asteraceae.^{20–22}

It is convenient to extend this list of classic CGA to include the galloyl conjugates of quinic acid and the cinnamoyl conjugates of quinic acid derivatives such as shikimic acid,^{23–26} methyl or butyl quinate,^{27,28} 4-deoxyquinic acid²⁹ and the recently characterised *muco*-quinic acid³⁰ which differs from the isomer described above by having an equatorial hydroxyl on carbon 3.

Other cinnamate conjugates

Cinnamic acids may be conjugated to many molecules other than quinic acid and its close relatives, although sometimes as very minor components of limited distribution. This second category includes:

- (1) esters of other hydroxy acids, particularly α -hydroxyhydrocaffeic, malic and tartaric but including galactaric, glucaric, gluconic, hydroxycitric, methoxyaldaric, phenylpyruvic and tartronic;
- (2) amides of amino compounds including aromatic amino acids, choline, anthranilic acids and diamines;
- (3) esters of polysaccharides, simple sugars, sugar alcohols including glycerol and *myo*-inositol, and glycosides including those of anthocyanins, flavonols and diterpenes;
- (4) esters of lipids including alkanols, alkandiol, ω -hydroxy-fatty acids and sterols; and
- (5) glycosides.

Cinnamate transformation products

There is little information on cinnamate transformation during food preparation and processing. It has been well established that cinnamic acids may be released from conjugates by hydrolysis and subsequently decarboxylated either by heat³¹ or microorganisms yielding various alkylphenols, but little consideration has been given to the possibility that food processing might generate novel transformation products. Possibly the first to be recognised was the grape reaction product (GRP) 2-*S*-glutathionylcaftaric acid and 2,5-di-*S*-glutathionylcaftaric acid formed from **caftaric** acid (caffeoyl tartaric acid) during wine making.^{32–35} Red wine contains adducts formed by the interaction of anthocyanins with vinyl phenol from *p*-coumarate decarboxylation^{36,37} and caffeic acid released during coffee roasting is partially converted to tetrahydroxy-phenylindans.^{38,39} These products will not be further discussed here as the cinnamate residues have been lost irretrievably.

Hydrolysis of CGA during coffee roasting also releases the 'typical' quinic acid residue (*vide supra*) which is then converted to the full theoretical complement of quinic acid and quinic lactone (quinide) diastereoisomers.^{40,41} Unhydrolysed CQA and FQA can be converted similarly to the corresponding 1,5-lactones or quinides^{42–44} and it seems possible that other diastereoisomers, either of the lactones or the original acids, might also be formed.

CINNAMATES IN FOODS AND BEVERAGES

Quantitative data are presented on a fresh weight basis unless specified otherwise.

Coffee

Coffee beans are one of the richest dietary sources of CGA and for many consumers must be the major dietary source. The CGA in coffee and coffee products have been extensively reviewed^{31,45} and only a summary is presented here. Depending on the species, green coffee beans contain some 6–10% CGA on a dry matter basis. During roasting there is a progressive destruction and transformation of CGA with some 8–10% being lost for every 1% loss of dry matter but substantial amounts survive to be extracted into domestic brews and commercial soluble coffee powders.

It has been estimated⁴⁶ that a 200 ml cup of roast and ground coffee might supply from 20 mg CGA (weak brew, very dark roast) up to 675 mg CGA (strong brew, very pale roast robusta). More recent studies (Clifford and Balyaya, unpublished data) suggest that 70–200 mg per 200 ml cup of arabica and 70–300 (or even 350) mg per 200 ml cup of robusta are more representative. Soluble coffee powder as sold in the UK during 1983–1984 (2 g per cup) might supply from 70 to 220 mg CGA consisting of 50–150 mg CQA, 4–21 mg FQA and 15–38 mg diCQA.^{31,47,48} Beverage prepared from Angolan robusta coffee beans, which have a unique composition, has been reported to contain some 12–44 mg litre⁻¹ caffeoyl-tyrosine and some 33–66 mg litre⁻¹ of a similar but unidentified conjugate.^{49–51}

The two major chlorogenic lactones (3-CQL and 4-CQL) are found at levels of 800–2400 and 200–1600 mg kg⁻¹ in roasted coffees.^{43,44} Coffee brew also contains traces of diterpene glycosides esterified with the relatively rare 3,4-dimethoxy-cinnamic acid.⁵²

Green and black tea

Tea leaf is the major dietary source of theogallin (5-GQA) and is accompanied by small amounts of *p*CoQA and CQA. These substances are not substrates for tea polyphenol oxidase and appear to survive fermentation and may thus be found in black tea as well as green tea. There are no data for their contents in tea brew, but 10–50 g kg⁻¹ has been reported for green and black tea leaf.^{53–56} Rooibos or bush tea (*Aspalathus linearis*), consumed in southern Africa, is quite different, but contains a novel conjugate between caffeic acid and the enol form of phenylpyruvic acid.⁵⁷

Maté

Maté is a traditional South American beverage prepared from the leaves of *Ilex paraguariensis* which is rich in CQA and diCQA. There have been few studies of the brew composition but green maté

material bought and brewed in Europe provided 107–133 mg CGA per approx 200 ml of which diCQA represented approximately one third. In contrast, roasted maté provided 16–41 mg CGA per approx 200 ml.⁵⁸ Components originally assigned tentatively as FQA are now thought to be caffeoyl-quinic lactones (Clifford, unpublished data). Mazzafera⁵⁹ doubted the authenticity of the plant material purchased in Europe and reported that when authentic material was brewed in the traditional manner (50–60 g leaf per litre boiling water), some 480–520 mg 5-CQA and 340 mg caffeic acid were extracted. However, due to significant differences in the methods of analysis, these data are not directly comparable with the former.

Cocoa

The caffeoyl amide of dihydroxyphenylalanine and the *p*-coumaroyl amide of hydroxyphenylalanine have been reported in cocoa.⁶⁰

Pome fruits – apples and pears

Apples are one of the more extensively studied fruits. CGA are found at similar concentrations in the isolated flesh and skin (mean values 30–60 mg kg⁻¹) but are absent from the seeds and pomace.^{61,62} Whole apples have been reported to contain 62–385 mg kg⁻¹ CQA (with 5-CQA always dominant), up to 40 mg kg⁻¹ *p*CoQA (with 4-*p*CoQA dominant in some cases) and smaller amounts of caffeoyl-, *p*-coumaroyl- and feruloyl-glucoses (up to 6, 6 and 9 mg kg⁻¹, respectively).^{63–65} Values of 100–200 mg kg⁻¹ total CGA on a dry matter basis are consistent with the foregoing.⁶⁶ It is clear that composition varies markedly with variety, cider varieties being richer than culinary. Commercial juices and ciders are also variable in composition with juices providing from undetectable up to 208 mg litre⁻¹ CQA^{65,67} and ciders 11–480 mg litre⁻¹ (Clifford and Mitchell, unpublished data) or even higher when produced from single varieties of apples (for example, >700 mg litre⁻¹ CQA and >400 mg litre⁻¹ *p*CoQA for cv Vilberrie).⁶⁸ Heat processing reduced the CGA content of apple juice significantly, especially when accompanied by treatment with pectic enzymes, but the fate of the cinnamates was not discussed.⁶⁹ The fruit of *Sorbus domestica*, used in Germany as an adjunct in cider making, contains some 1500 mg kg⁻¹ CQA.⁷⁰

According to some investigators^{63–65,67} pears and pear juices are similar in composition with some 60–280 mg kg⁻¹ CQA in whole fruit and up to 240 mg litre⁻¹ in juice but others^{71–73} have also reported the presence of caffeoyl- and *p*-coumaroyl-L(–) malic acids (ie 2*S* malic acid) and 3,5-diCQA (1–8 mg kg⁻¹).

Stone fruits

Stone fruits contain CQA and *p*CoQA with mean contents in the range 150–600 mg kg⁻¹ and 3–

200 mg kg⁻¹, respectively, at least in cherries found mainly adjacent to the peel. Compared with apples and pears, the 3-isomers are more prominent relative to the 5-isomers. Cherries and plums are rich in *p*CoQA compared with peaches and apricots.⁶⁴ Studies on commercial juices and nectars (containing added sugar syrup)⁶⁵ are not entirely in agreement with the foregoing. Levels of 5-CQA were some 3–4 × greater than 3-CQA in peach and apricot juice with total CQA reaching ≈9 and ≈4 mg litre⁻¹, respectively: *p*CoQA was not detected but some 0.5 mg litre⁻¹ of free cinnamic acids was noted. Heat treatment of peach juice caused significant losses of CQA and *p*CoQA especially when combined with pectic enzyme treatment.⁶⁹

Berry fruits

Berry fruits encompass several distinct botanical families and thus differ markedly in their composition. Relatively few quantitative data are available. Blueberries contain 0.5–2 g kg⁻¹ CQA^{74,75} and only small amounts (≈60 mg kg⁻¹ in total) of other conjugates, mainly glucosides. Blackcurrants supply some 140 mg kg⁻¹ of total conjugates in which CQA dominate (≈40%), *p*CoQA and caffeoyl-glucose account for some 15% each and feruloyl-glucose, *p*-coumaroyl-glucose and total cinnamate β-D-glucosides for some 8% each. Blackberries provide some 70 mg kg⁻¹ with CQA dominant. Raspberries, strawberries, redcurrants and gooseberries are poor sources by comparison with the berries mentioned above, providing mainly glucose esters and β-D-glucosides totalling some 20–30 mg kg⁻¹.^{74,76} Cranberries contain a complex series of sugar derivatives involving both ester and glycosidic linkages, sometimes both, and varying numbers of sugar residues.⁸ Bilberry wine has been reported to contain 50 mg litre⁻¹ of CGA.⁷⁵

Citrus fruits

Citrus fruits contain the comparatively rare conjugates of glucaric (feruloyl, *p*-coumaroyl and diferuloyl) and galactaric acids (feruloyl, *p*-coumaroyl) and some associated lactones. These occur mainly in the peel (170–250, 27–62, 55–67 mg kg⁻¹ in oranges, grapefruit and lemon respectively), and at ≈25, ≈17 and ≈2 mg kg⁻¹ in the flesh.^{77,78} A range of other conjugates, involving sugars and anthocyanins, have also been reported.

The following data relate to the total cinnamates released by hydrolysis. The flavedo of oranges (and juice) supplied the following in bound form: ≈30 (8–18) mg kg⁻¹ sinapic, ≈130 (40–50) mg kg⁻¹ ferulic, ≈18 (8–12) mg kg⁻¹ *p*-coumaric and ≈4 (11–25) mg kg⁻¹ caffeic for which there is known to be a low recovery: the corresponding data for grapefruit were ≈50 (12–20), ≈152 (30–40), ≈72 (15–20) and ≈4 (5–8) mg kg⁻¹.^{79,80} Juice from blood oranges contains these same cinnamates at a total concentration in the range 50–160 mg litre⁻¹ again with ferulic

acid (30–64 mg litre⁻¹) dominant.⁸¹ Some workers have reported up to 2 mg litre⁻¹ 5-CQA and smaller amounts of free cinnamic acids and feruloylglucose in commercial orange juices and nectars (containing added sugar syrup).⁶⁷

Grapes and wines

Grapes are characterised by *cis*- and *trans*-cinnamoyltartrates (ie esters of L(+)-tartaric acid, ie 2R3R tartaric acid), in the pulp and juice as well as the skin, and the classic chlorogenic acids are absent.⁸² As usual caffeoyltartaric (caftaric) acid dominates but is accompanied by *p*-coumaroyltartaric (coutaric), feruloyltartaric (fertaric) and *trans*-coutaric glucoside.⁸³ The composition depends on the species and cultivar to a greater extent than season, ripeness and growing location, and may serve as a chemotaxonomic criterion^{84,85} but literature values vary considerably. Grape juice from red and white varieties of *Vitis vinifera* grown in America varied from ≈10 to 430 mg litre⁻¹ caftaric in one study but up to 600 mg litre⁻¹ in another, with little variation between skin (150 mg kg⁻¹) and flesh (110 mg kg⁻¹) although the skin is usually richer: coutaric ranged to 55 mg litre⁻¹ and fertaric to 16 mg litre⁻¹.^{65,67,85–87} Levels in grapes from Tuscany were in the range 2–7 mg kg⁻¹ caftaric, 0.4–5 mg kg⁻¹ coutaric and up to 3 mg kg⁻¹ fertaric.⁸⁸ Juice from *V. aestivalis* and *V. labrusca* contained 1350 and 460 mg litre⁻¹ caftaric respectively, whereas it was barely detectable in juice from *V. rotundifolia*.⁸⁵

Levels of caftaric and coutaric decline markedly by oxidation during the wine-making process and become more variable but levels of fertaric change less. Wines are extremely variable, with contents increasing with time of skin contact.^{89,90} Some American wines contained 9–116, up to 9 and up to 16 mg litre⁻¹ for caftaric, coutaric and fertaric respectively. Some 43 mg litre⁻¹ caftaric was reported for some French wines,^{86,91} but only 2–12 mg litre⁻¹ for some Spanish wines.^{92–94} Japanese Koshu wines contained appreciably higher concentrations of caftaric (40–304 mg litre⁻¹) and coutaric (28–127 mg litre⁻¹) although Japanese Semillon wines were very similar in this respect to French Semillon.⁹⁵

2-S-Glutathionylcaftaric acid has been reported in juices and wines at 6–8 mg litre⁻¹.^{65,93} Prolonged maturation can result in release of up to 3 or 4 mg litre⁻¹ caffeic and *p*-coumaric acid by hydrolysis, and ethyl esters may form by transesterification.^{32–35,83,95} Red grapes also contain several 6-*O*-*p*-coumaroyl- and caffeoyl-glucosides of various anthocyanidins at levels which vary considerably with cultivar.^{88,96,97}

Other fruits

Pineapple is characterised by the presence of the caffeoyl- and *p*-coumaroyl-glycerols but no quanti-

tative data are available.⁵ Other workers have reported small amounts of free cinnamic acids, 5-*p*CoQA and feruloylglucose totalling about 3 mg litre⁻¹.⁶⁷ Some dates contain caffeoylshikimic acids.^{24,25} Kiwi fruit contains ≈ 11 mg kg⁻¹ conjugated caffeic acid, about a quarter of which is fibre-associated.⁹⁸

Brassica vegetables

3-CQA, 3-*p*CoQA and 3-FQA have been reported^{99,100} in the leafy Brassicas (kale, cabbage and Brussels sprouts) at levels of 6–120 mg kg⁻¹, up to 104 mg kg⁻¹ and up to 37 mg kg⁻¹ respectively along with smaller amounts of the 4-isomers and little or none of the 5-isomers. Significant amounts of sugar derivatives have also been reported with feruloyl glucose and sinapoyl glucose dominating and levels up to 350 mg kg⁻¹ in kale and up to 80 mg kg⁻¹ in the others and, in red cabbage and radish, cinnamoyl esters of anthocyanins. Broccoli contained about 60 mg kg⁻¹ CGA plus about 20 mg kg⁻¹ sugar esters, but cauliflower and radish contained not more than 20 mg kg⁻¹ of CGA and sugar esters combined. More recent studies¹⁰¹ indicate that some broccoli florets contain several mixed feruloylsinapoyl esters of gentiobiose totalling >300 mg kg⁻¹. Radish leaf contained a series of malic acid esters at levels totalling 240–550 mg kg⁻¹. The corresponding levels in tuber peel were 40–120 mg kg⁻¹ but only 2–23 mg kg⁻¹ in peeled tubers. Only traces of sugar and quinic acid esters were found.¹⁰² Sinapoyl-choline is a characteristic component of mustard seed (*Sinapis alba* and *Brassica nigra* = *B. juncea*), mustard and mustard containing products but quantitative data are not available. Small amounts (up to 3 mg kg⁻¹) were found in some cabbages.⁹⁹

Chenopodiaceae – spinach and beetroot

Spinach is dominated by *p*-coumaroyl-*meso*-tartaric acid at levels around 200 mg kg⁻¹ along with *p*-coumaroylmalic acid (25–30 mg kg⁻¹) and glucose esters totalling some 60–80 mg kg⁻¹. Beetroot root provides traces of quinic acid and glucose esters and the leaf slightly larger amounts of the glucose esters but the tartaric acid derivative found in spinach leaf was not detected.^{103,104} Cell wall-bound cinnamates are dealt with under fibre, *vide infra*.

Asteraceae

The Asteraceae is a very large group of plants which vary markedly in their profiles and contents of cinnamates. In some cases the profile does not differ with tissue for a given species (for example dandelion¹⁰⁵) but it should not be assumed that this is always the case.

The leaves of lettuce, endive and chicory provide quinic acid, malic acid and tartaric acid esters among which caffeic derivatives, especially dicaffeoyltartaric acid (chicoric acid), dominate. The total cinnamate contents were, respectively, 50–120 mg kg⁻¹, 200–500 mg kg⁻¹ and some 20 mg kg⁻¹.¹⁰⁴ CQA and

diCQA have been found in chicory root (total 2.6 g kg⁻¹) and in dandelion and chicory root-based coffee substitutes (some 100–400 mg kg⁻¹).^{106,107} Although these workers saw no evidence of mono- or di-caffeoyltartaric acid in the coffee substitutes these have been found throughout all parts of dandelion.¹⁰⁵ Another study found caffeoylshikimic acid but not the quinic acid esters referred to above in endive.²⁶

Artichoke (*Cynara scolymus*) and scorzonera provide some 450 and 180 mg kg⁻¹ CQA respectively with little or none of the other conjugates.¹⁰⁰ Artichoke is characterised by significant amounts of 3,5-diCQA and 1,5-diCQA.^{108–110} These isomers are not easily resolved and 1,5-diCQA is unstable in aqueous solutions, isomerising rapidly to 1,3-diCQA, otherwise known as cynarin(e). These factors may account for Herrmann⁸⁶ failing to mention these isomers although cynarin forms 800–1300 mg kg⁻¹ dry basis of the capitula.¹¹¹ It is not known whether any cinnamates occur in the artichoke-based beverage marketed as Cynar, but cynarin is found in some herbal medicines.¹¹² The relatively scarce triCQA, tetraCQA and caffeoylsuccinoyl-quinic acids (*vide supra*) have been reported in Asteraceae used as herbal medicines. These conjugates have not been reported in food plants other than burdock (*Arctium lappa*),^{20,21} an extract of which was once used in preparing the cordial 'Dandelion and Burdock'. Absinthe (*Artemisia absinthum*), tarragon (*A. dracunculus*) and mugwort (*A. vulgaris*) contain significant amounts of imperfectly defined quinic acid esters of caffeic acid¹¹³ but it is not known if these are transferred to vermouth and similar beverages in which extracts of absinthe are traditionally used.

Solanaceae

Potato tubers provide some 500–1200 mg kg⁻¹ dry basis total CGA^{114,115} (mainly CQA plus a little diCQA¹¹⁶) at harvest and the content rises slowly on storage especially in the light¹¹⁷ and during wound healing.¹¹⁸ These are concentrated in the peel where they may reach levels of 2–5 g kg⁻¹ (Ref 119) or even higher.¹²⁰ *p*-Coumaroyl conjugates of anthocyanins are present in peel (up to 7 g kg⁻¹) and flesh (up to 2 g kg⁻¹).¹¹⁹ Bell peppers contain caffeoyl and feruloylputrescine in the placenta during fruit development but only small amounts of glucose derivatives in mature fruit. Aubergines have a significant CQA content (600 mg kg⁻¹) and small amounts of FQA and anthocyanin conjugates (15 mg kg⁻¹). During the ripening of tomatoes, CQA in the pulp are partially replaced by glucosides in the peel – ripe fruit contain some 10–80 mg kg⁻¹ CQA, 5–15, 15–50 and 20–70 mg kg⁻¹ glucosides of ferulic, caffeic and *p*-coumaric respectively, plus up to 20 mg kg⁻¹ *p*-coumaroyl-glucose.^{104,116,121} Caffeoylglucaric acid is found in tomato leaf.¹²² It has not been possible to trace any compositional data for tomato juice or purée.

Apiaceae

Celery contains a modest but variable amount of CQA ($2\text{--}65\text{ mg kg}^{-1}$), *p*CoQA (up to 3 mg kg^{-1}) and FQA (up to 8 mg kg^{-1}),^{123,124} as do carrots ($20\text{--}120\text{ mg kg}^{-1}$)¹⁰⁰ but it should be noted that seedsmen attribute the root fly-resistance of modern varieties to the virtual absence of CGA.

This botanical group also supplies a range of spices and some of these have substantial contents of quinic acid conjugates (predominantly CQA but with appreciable FQA and *p*CoQA) and reaching 3 g kg^{-1} in the case of anise, just over 2 g kg^{-1} in fennel, approaching 2 g kg^{-1} in caraway, but only some 200 mg kg^{-1} in coriander.¹²⁵

Lamiaceae and Boraginaceae

The Lamiaceae supply many leafy herbs (basil, marjoram, melissa, oregano, peppermint, rosemary, sage, spearmint and thyme) characterised by the occurrence of rosmarinic acid, the caffeic acid conjugate of α -hydroxyhydrocaffeic acid, at concentrations ranging from $1\text{ to }17\text{ g kg}^{-1}$ dry basis¹¹³ or even in excess of 20 g kg^{-1} (dry basis) in some samples of basil and rosemary.^{126,127} A smaller quantity (500 mg kg^{-1} dry basis) has been found in borage.¹¹³ Melissa also contains a series of dimeric and trimeric caffeic acid conjugates.¹²⁸ It has not been possible to trace data for mixed herbs, stuffings, forcemeats, etc.

Fabaceae – legumes and pulses

There have been various reports of cinnamates in legumes and pulses^{23–26,129} but the distribution is variable across tissues, at least in Faba (*Phaseolus* spp) type beans where the largest concentrations of L(–)-malic acid conjugates (phaseolic acids) are found in leaves, with moderate but variable contents (up to 100 mg kg^{-1}) in pods and traces only in the seeds.¹⁰⁴ Peas contain *p*-coumaroyl, caffeoyl and feruloyl derivatives of flavonol glycosides¹³⁰ and lentils 0.1 mg kg^{-1} of bound *p*-coumaric acid.⁹⁸ Caffeoyl and *p*-coumaroyltartronic acids have been found in dry mung beans (*Vigna radiata* = *Phaseolus aureus*).¹³¹

The novel compound, 1-caffeoyl-4-deoxyquinic acid, along with 3-CQA and 5-CQA, has been reported in the foliage of a wild groundnut (*Arachis paraguariensis*) but it is not clear if these compounds occur also in the edible portions of the *A hypogaea*.²⁹ Commercial peanut flour and protein isolates have significant cinnamate contents including $500\text{--}1350\text{ mg kg}^{-1}$ *p*-coumaric acid.⁶⁹

Miscellaneous vegetables

Corn salad (Valerianaceae) contains approximately 1 g kg^{-1} CQA. Rhubarb petiole (*Rheum* spp, Polygonaceae) contains traces of glucose esters but the leaf contains *p*CoQA as the only CGA. Leeks (Alliaceae) contain small amounts of FQA (up to 12 mg kg^{-1}) without detectable CQA.¹⁰⁰ The spice star anise contains from $100\text{ to }200\text{ mg kg}^{-1}$ CGA in which, surprisingly, 4-*p*CoQA dominates at 52--

125 mg kg^{-1} .¹²⁵ Sweet potatoes (*Ipomoea batatas*) contain CQA and diCQA in the range $100\text{--}500\text{ mg kg}^{-1}$,¹³² a series of long chain (C_{16} , C_{17} , C_{18}) alkyl ferulates¹³³ and in pigmented varieties caffeoyl derivatives of anthocyanins.¹³⁴ Red-pigmented yams (*Dioscorea* spp) contain sinapic esters of a cyanidin glycoside.¹³⁵ Chinese water chestnuts (*Eleocharis dulcis*, Cyperaceae) contain cell wall-bound cinnamates, *vide infra*.

Cereals and cereal products including beers and bran

It has not been possible to trace any data for bread, biscuits, cakes, breakfast cereals, etc.

Conjugates of cell wall polysaccharides

Cereals are characterised by cinnamic acids, especially ferulic acid, esterified to arabinose residues in primary cell wall arabinoxylan in the aleurone layer and pericarp. A portion of this total cinnamate exists as ferulate dimers linked in various ways including 5,5' or 5,8' carbon-carbon bonds.^{136–138}

Barley bran contains $\approx 50\text{ mg kg}^{-1}$ bound ferulic, $\approx 30\text{ mg kg}^{-1}$ bound *p*-coumaric and 3 mg kg^{-1} diferulic acid. The endosperm content is $\approx 3\text{ mg kg}^{-1}$ total and the aleurone is intermediate.⁶⁹ Rice endosperm cell walls contain 12 g kg^{-1} esterified cinnamic acids comprising $\approx 9\text{ g kg}^{-1}$ ferulic, $\approx 2.5\text{ g kg}^{-1}$ *p*-coumaric and $\approx 0.5\text{ g kg}^{-1}$ diferulic esters.¹³⁹ While whole wheat contains some $20\text{--}30\text{ mg kg}^{-1}$ cinnamic acids esterified to polysaccharides,¹⁴⁰ the derived wheat bran contains some $4\text{--}7\text{ g kg}^{-1}$ and maize bran as much as 30 g kg^{-1} .^{140–142} Maize leaves contain conjugates of hydroxycitric acid.¹⁴³

Water chestnuts (*Eleocharis dulcis*, Cyperaceae) are botanically closer to the cereals than to other common fruits and vegetables and are characterised also by a significant content of cell wall-bound cinnamates ($>7\text{ g kg}^{-1}$ ferulate and $>4.5\text{ g kg}^{-1}$ diferulate, the majority of which is 8-*O*-4 linked).¹⁴⁴

Spinach and sugar beet contain cell wall polysaccharides which are esterified with *p*-coumaric and ferulic acids, and to a lesser extent with what is probably cross-linked ferulate dimer.^{142,145} Sugar beet fibre contains some 9 g kg^{-1} of ferulic acid attached to galactose or arabinose residues in pectic side-chains. Digestion, using an *in vitro* model of the human colon suggests that some 90% of cinnamates are released from sugar beet fibre in 24 h compared with some 35–40% from wheat bran but the released cinnamate may be further metabolised by the gut flora and its bioavailability is uncertain.¹⁴²

Other conjugates and free cinnamates

Wheat, maize and rye contain ferulic and *p*-coumaric esters of sterols and stanols.^{146,147} The leaves of rye contain feruloyl esters of gluconic and methoxyaldaric acids.¹⁴⁸ Oats contain a series of 24 caffeic and ferulic esters of glycerol, long chain alkanols, alkandiol ($n = 22, 23, 24$) and ω -hydroxy acids

($n = 26, 28$).^{149–153} In addition, there are more than 25 compounds which are esters of anthranilic acid or 5-hydroxyanthranilic acid with either *p*-coumaric, caffeic or ferulic acids (avenanthramides) or with their ethylenic analogues (avenulamides).^{154–156} Oat meal has been reported to contain some 200–300 mg kg⁻¹ of esterified ferulic acid.¹⁴⁰

Barley contains small amounts of free cinnamic acids, mainly *p*-coumaric and ferulic which together range from 8 to 26 mg kg⁻¹ (Ref 157) but this value rises during malting (18 mg kg⁻¹). The content rises further on mashing, presumably by hydrolysis of bound forms, with ferulic acid dominating (115 mg kg⁻¹) and lesser but equal amounts of caffeic and *p*-coumaric making up the total (167 mg kg⁻¹). A limited range of beers (including lagers, stouts, ales, etc) contained 2–8 mg litre⁻¹ but German 'rauchbier' reached almost 20 mg litre⁻¹.¹⁵⁷

DIETARY BURDEN

A major objective of this review, as originally conceived, was to identify the commodities most likely to provide the greatest amount of (i) a particular cinnamate (independent of the conjugate type and extent of hydrolysis in the gastro-intestinal tract) and (ii) of a particular conjugate. These two approaches have been taken since it seems possible that the form of the conjugate might influence absorption as is suggested in the case of flavonol glycosides.¹⁵⁸ With the exception of some beverages, the lack of data for the composition of commodities as consumed, ie the *edible portion after processing, cooking, baking, etc*, effectively make such an assessment impossible. In the circumstances, it is possible only to highlight the sources which have the *potential in normal usage to make a greater contribution than most*.

The values for burden set out below have been calculated from representative but rounded quantitative data presented in the first part of this review and assuming, for simplicity, that a serving of fruit or vegetable is 100 g, of non-alcoholic beverage is 200 ml, of cider or beer is 500 ml, of wine is 100 ml, of bran is 10 g and of herbs is 1 g.

There is no doubt that caffeic acid is the cinnamate which occurs most extensively, and the various CQA and diCQA are the most ubiquitous conjugates. Usually the 5-isomer dominates, but in stone fruit and brassicas it is the 3-isomer. Because of the quantity commonly consumed, coffee beverage must top the list with 200 ml instant brew (2% w/v) supplying 50–150 mg CQA. The theoretical yield of caffeic acid would be ≈ 25 –75 mg. Blueberries, aubergines, apples, cider and green maté could be important in some populations. Sweet cherries and Savoy cabbage along with apples, apple juice and cider probably supply the majority of the *p*CoQA while coffee must, in the majority of cases, come first for conjugated ferulic acid, followed by citrus juices.

In view of the quantities consumed by some populations, wines may be the major source of the tartaric

acid conjugates, but the literature data are extremely variable probably reflecting differences in wine-making practice across the world. Clearly grapes and grape juice also could make a significant contribution especially of caftaric at up to 60 mg per 200 ml juice. Lettuce, with 5–15 mg 100 g⁻¹, will be the major source of chicoric (dicafeoyltartaric) acid in the UK, but endive may have twice the concentration. Spinach will be the major source of coutaric (albeit *meso*-tartaric) and is almost certainly the richest source of conjugated *p*-coumaric acid at some 30–35 mg per 100 g.

Lettuce, since it is eaten raw, will probably be the main UK source of caffeoylmalic acid (up to 3 mg 100 g⁻¹) but spinach may be richer. Legumes might also be an important source but the quantitative data are inadequate.

Broccoli florets and leafy cruciferous vegetables will be the major source of sugar esters and of conjugated sinapic acid – broccoli florets supplying >10 mg bound sinapic acid per 100 g. Tomatoes and tomato products are likely to be the major source of glucosides at up to 13 mg 100 g⁻¹ in total, and possibly the second richest source of conjugated *p*-coumaric acid at ≈ 3 mg 100 g⁻¹.

Herbs are the only source of rosmarinic acid. While some are very rich, the levels consumed make this a comparatively poor source of conjugated caffeic acid, generally supplying only low milligram quantities per gram, but up to ≈ 10 mg in some cases.

Cereal bran and bran-enriched products must be the most important source of cell wall-bound cinnamates. Even with small servings, say 10 g, maize or wheat bran could supply up to 30 and 7 mg of ferulate respectively making such products the richest dietary source of this cinnamate although its bio-availability after release in the colon is unclear. However, coffee brew could supply up to 10 mg ferulate (as FQA) per 200 ml cup so, for many people, coffee will be the major source.

There are insufficient quantitative data to allow useful comments to be made about the other conjugates.

CONCLUDING REMARKS

Cinnamate content of foods and beverages is incompletely known yet variable, as are diets. For a meal consisting of two glasses of wine, salad-based starter, main course with potatoes and two other vegetables including broccoli or spinach, a blueberry sweet, plus two large cups of strong coffee, the intake might be as much as 1 g of conjugates (predominantly CGA) or 500 mg of cinnamates (predominantly caffeic). The coffee alone would supply approximately 70% of the total *even if there were no losses in cooking*. In contrast, breakfast consisting of two slices of white toast and a cup (or two) of tea would supply hardly any cinnamates, but replacing white bread with wholemeal, taking a bran-containing cereal and

a glass of citrus juice might supply up to some 60 mg cinnamates (there are inadequate data for conjugates contents) in which ferulate would dominate.

It seems likely that the UK population will fall into several categories depending on (i) their consumption of coffee, (ii) their consumption of bran, and (iii) their consumption of citrus. Those who drink several cups of coffee per day augmented by bran and citrus might easily achieve 500–800 mg cinnamates (or even 1 g for the highest coffee consumption) whereas those who eschew all these and take little fresh fruit or vegetables might struggle to achieve 25 mg.

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Caftaric acid in grapes and conversion to a reaction product during processing

by

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L'acide caftarique des raisins et sa conversion en produit de réaction au cours de la vinification

Résumé. — A l'aide de HPLC il a été mis en évidence que l'acide caftarique (caffeoyl tartarique) subit des pertes rapides lors du foulage des raisins dans le cas où ceux-ci ne sont pas complètement protégés contre l'oxydation, comme par ex. foulage en atmosphère inerte en présence d'un niveau élevé de métabisulfite de potassium et d'acide ascorbique. En l'absence de telles précautions les valeurs de la teneur originelle en acide caftarique, considérées comme étant caractéristiques des variétés de vigne, sont douteuses. Des échantillons totalement protégés de 5 variétés blanches et 3 variétés rouges de *Vitis vinifera*, mûres, se rangent entre 200 mg/l de jus pour Grenache et 50 mg/l pour Carignane. Dans la mesure que l'exposition à l'oxygène et l'activité de phénolase sont évaluables la perte peut être importante. Une majeure partie de cette perte consiste dans un produit de réaction de l'acide caftarique ici pour la première fois décelé (CRP). Ce CRP peut pénétrer dans le vin et sa détermination par HPLC ainsi que celle de l'acide caftarique non transformé montrent, en fonction de la variété de vigne, une grande conversion au cours de la préparation normale du moût. De telles analyses de vins peuvent servir d'indicateurs de l'exposition à l'oxygène au cours de la préparation du moût. La nature du CRP est actuellement étudiée et sa présence peut contribuer à expliquer la faible récupération de l'acide caftarique du moût ou du vin.

Introduction

Caftaric acid (caffeoyl tartaric acid) and its relatives, coumaric and fertaric acids (*p*-coumaroyl and feruloyl tartaric acids), make up a major portion of the phenolic substances of fresh grape juice and wines made from it (ONG and NAGEL 1978, SINGLETON *et al.* 1978). They are major phenolic components, oxidation substrates, and browning precursors in wines made without appreciable pomace contact. It is therefore important to know the true content of these substances in grapes and understand their modification during normal processing.

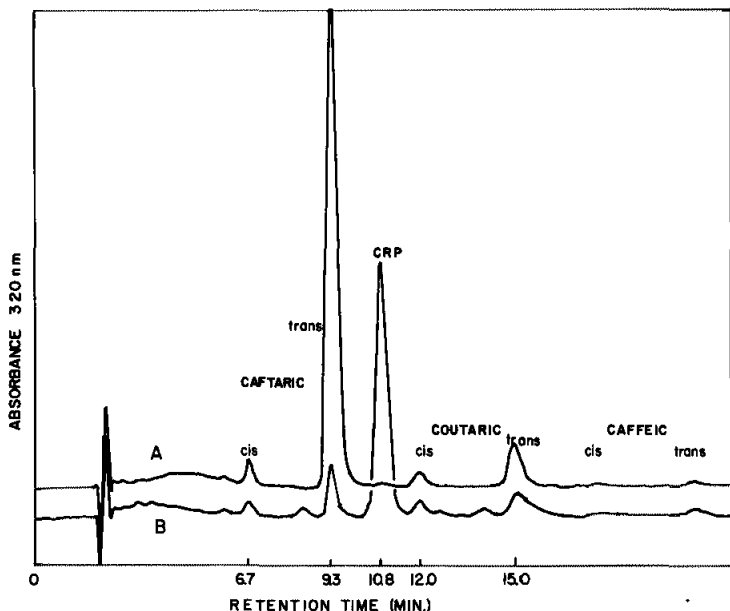
The content of caftaric acid reported in various samples has been quite variable, ranging from tens of mg/l to more than three hundred. Part of the variation is varietal, some can be weather or ripeness related, but part appeared to be technique and sample preparation. Our previous values for *trans*-caftaric acid content by HPLC averaged lower on wines than on the corresponding musts and were generally lower on musts than others had reported in grapes (SINGLETON and TROUSDALE 1983). NAGEL *et al.* (1979) reported that wines retained less than half of the caftaric and coumaric acids of their musts. MYERS and SINGLETON (1979) examining the nonflavonoids of white wine found the situation complex. A considerable portion of the 320 nm absorbance characteristic of hydroxycinnamates was due to highly polar unknown compounds that resembled products from chlorogenic acid under certain conditions of enzymic oxidation. Whether these were artifacts remained uncertain.

Isolation of caftaric acid from California juices and wines prepared under conditions similar to good commercial practice produced much lower recovery than the same isolation procedure applied to an English experimental wine (SINGLETON *et al.* 1978). Extraction with solvents from acidified musts, even with ethanol after saturation with ammonium sulfate (SINGLETON 1961), recovered much less of the compound from the sample than expected from the characteristics of caftaric acid.

To better explain these observations, we wished to determine the original content of caftaric acid in the grape and to investigate the size and nature of the losses incurred in normal processing.

Materials and methods

The grapes were harvested directly from the University vineyards and sampled by carefully snipping with small scissors at the torus without damaging the berry skin. The whole group of clusters being processed to wine was sampled with several hundred



HPLC chromatograms of: A — fully protected fresh Chenin blanc juice, B — the same juice unprotected from air oxidation during crushing.

Chromatogrammes HPLC de: A — jus frais de Chenin blanc totalement protégé, B — même jus non protégé contre l'oxydation pendant le foulage.

berries being collected, a few at random from about every fifth cluster. The berries were gently mixed, subsamples counted, weighed, and processed immediately.

Berry subsamples (100 berries as a rule) were placed in a glove box under an atmosphere of CO₂ flowing in at a high rate and verified as essentially oxygen-free by an oxygen electrode system. Two to four subsamples of berries from each variety were processed in the glove box entirely to the stage of filtered juice ready for HPLC. To the 100 berries were added 1 g of potassium metabisulfite and 1 g of ascorbic acid. Levels of 100 mg of each agent seemed about adequate, but to ensure maximum effect and since excess had no apparent deleterious effect on chromatography, the larger amounts were used. The mixed mass was crushed and pressed in cheesecloth by means of a hand-operated press as quickly, completely and uniformly as possible. The juice was measured and a portion filtered for HPLC through a Millipore 0.45 µm membrane filter. Meanwhile, other berry subsamples were also prepared with and without additions of ascorbic acid and bisulfite but in air. One set was unprotected and the crushed grapes exposed to air for 15 min to 1 h.

HPLC was carried out on each juice sample as soon as possible, although once protected by high SO₂ and ascorbic acid and filtered further change was slow. The HPLC apparatus was a Waters Associates system including a 720 controller, 730 data module, M6000 A pumps, a 710B autoinjector and a Perkin-Elmer LC-55B variable wavelength detector. The effluent was monitored at 320 nm and known dilutions of *trans*-caftaric acid were used to determine the response factor (unit mass per unit peak area) and thus to calculate the amount of caftaric acid or derivatives in each peak. The column was reversed phase, C18 (5 µm packing), 4.6 × 250 mm, and protected with a guard cartridge of the same packing (Brownlee Labs., Inc.). Isocratic development was with 0.05 M ammonium dihydrogen phosphate made to pH 2.6 with phosphoric acid, 16 % methanol at 1.5 ml/min and ambient temperature. Direct injections, 50 µl, were made of the closely filtered sample along with comparable injections, 30 µl, of a standard solution of known caftaric acid.

The commercial-style musts were prepared by passing the grapes through a small Garolla-type crusher-destemmer, making the must to 50 ppm in SO₂, and pressing lightly. The musts were allowed to settle in a 0 °C room overnight and the supernatant juice sampled to compare with the sample taken at pressing. A second portion of the whole must (pomace included) was fermented 5 d and then pressed and sampled as wine.

Results and discussion

There are three obvious possible sources of loss of *trans*-caftaric acid during processing — oxidation, hydrolysis, and *cis*-isomerization. Oxidation will be shown to be rapid and important. Hydrolysis, unless external pectinase or other esterase has been added, is relatively slow and usually minor. Even at equilibrium *cis*-caftaric acid content is small.

Caftaric acid content is maximal and should represent the true content in fresh juice of the grape berry when processing is cool, rapid, with minimal light, and fully protected from oxidation, i.e., in inert atmosphere, immediately both high SO₂ and ascorbic acid to maintain reducing conditions. Under such conditions a typical HPLC chromatogram (Fig.: A) shows *trans*-caftaric acid, *trans*-coutaric acid and traces of their *cis*-forms. In time some caffeic acid, both *trans* and *cis*, slowly appears.

The figure (B) shows a typical HPLC chromatogram of the same juice after incomplete protection from enzymic oxidation. Note that the major change is the production

Table 1
 Caftaric and coutaric acid content of juice and berries well protected from oxidation
 Teneur en acides caftarique et coutarique de jus et baies bien protégés contre l'oxydation

Variety	Vintage	Caftaric acid						Coutaric acid		
		100-berry replicates, mg/l juices						mg/kg ¹⁾ fresh berries	mg/l juice Mean	mg/kg ¹⁾ fresh berries
		A	B	C	D	Mean	SO ₂ + Asc. no CO ₂			
Carignan	1983	47	51	52	49	50	53	41	11	9
Chardonnay	1983	207	196	182	180	191	197	158	20	16
Chenin blanc	1982	—	—	—	—	74	13	62	—	—
Chenin blanc	1983	102	100	98	98	100	90	82	7	6
French Colombard	1982	—	—	—	—	105	87	88	—	—
French Colombard	1983	76	76	76	77	76	78	64	11	9
Grenache	1983	190	201	202	210	201	193	158	16	13
Ruby Cabernet	1983	63	66	67	70	67	67	55	13	11
Semillon	1982	—	—	—	—	142	119	118	—	—
Semillon	1983	92	92	90	86	90	79	74	3	2
Thompson Seedless	1982	—	—	—	—	112	85	99	—	—
Thompson Seedless	1983 a	62	65	64	64	64	41	55	2	2
Thompson Seedless	1983 b	—	—	—	—	—	71	62	7	6

¹⁾ Calculated assuming all berry fluid has the same content as the best-protected expressed juice, subtracting an approximate value (10 % for seeded, 5 % for unseeded) for dry, nonsugar solids, and using Brix density to convert from volume to weight.

of a new symmetrical peak at a retention time of 10.8 min representing caftaric reaction product (CRP). Based upon retention time under identical conditions the same CRP is produced in all grape varieties tested. It is called caftaric reaction product because it was possible to produce additional product from added caftaric acid and grape phenolase. Furthermore, the relative amounts of the remaining *trans*-caftaric acid and the CRP are reciprocal, i.e., the less the caftaric acid is protected from oxidation the more CRP appears and *vice versa*. In fact, the sum of the percentage contribution of these two components to the total remaining 320 nm absorbance was nearly constant and caftaric acid and CRP inversely proportional.

The means of triplicate HPLC determinations of *trans*-caftaric and *trans*-coutaric acids on protected, replicate, 100-berry samples are shown in Table 1 for grape varieties harvested in 1983 and a few protected samples from 1982. The ultraviolet absorption of CRP is similar to that of caftaric acid with a maximum at 325 nm in 0.1 N formic acid in 20 % methanol. Therefore, amounts of CRP calculated from the caftaric acid response factor should be meaningful. A separate response factor was used to calculate *trans*-coutaric acid content. The replicate analyses were highly reproducible (coefficient of variability averaged 1.6 %) and the 100-berry samples only slightly more variable. This gives additional confidence that the maximum caftaric acid contents indicated are good estimates of the original berry content. Other factors studied were the reproducibility of injection and of the response factor. It was found that the amount injected and the preparation of the standard could easily produce variation in response factor sufficient to vary the calculated caftaric acid content by a factor of 2. Weighing a relatively large amount (100 mg) of pure caftaric acid to make the standard solution, preparation of dilutions to a similar level as the samples being measured (about 50—100 mg/l), and use of similar amounts per injection (not less than 10 μ l per injection) were found preferable and used to develop the 1983 data in Table 1. The 1982 data are perhaps less reliable because the techniques were under development and less replication was used.

There is apparent loss of caftaric acid to other products because the sum of the remaining *trans*-caftaric acid plus CRP is appreciably lower in most cases than the original *trans*-caftaric acid content (Table 2). This loss presumably results from conversion of caftaric to additional products sufficiently changed in spectral properties or too numerous to be detected as significant peaks. (Note extra peaks in the figure, B.) Hydrolysis and *cis*-isomerism could contribute. Nevertheless, the CRP represents in most instances a very sizable fraction of the remaining 320 nm absorbance and a major fraction of the original *trans*-caftaric acid content. In no instance was CRP detected in juices well protected from oxygen (Table 2).

Coutaric acid appeared more readily retained than caftaric acid. Little or none was lost in the semi-protected laboratory juice samples, but in unprotected samples the losses were: 26 % for Chardonnay, 13 % for Chenin blanc, and 53 % for Semillon. The rate of loss of caftaric acid was rapid, being considerable by 10 min and approaching completion by 1 h after exposure to air by unprotected crushing. CRP was higher after about 15 min than after longer oxidation. It seems again clear (Table 2) that the caftaric acid content of grapes and wines represents a component of the easily expressed vacuolar juice and is not contributed appreciably from the firmer tissues, in sharp contrast to anthocyanins and other flavonoids. The slightly higher content of caftaric acid in wine fermented on the pomace than corresponding wine from juice is attributed to less complete oxidation in the former.

Discovery of this CRP entity is considered exciting and details of its characteristics and identity will be the subject of following reports. It appears to help explain a number of observations. Previously we had been able to recover about half or less of the

Table 2

Loss of *trans*-caftaric acid and generation of CRP by storage and processing
 Perte en acide *trans*-caftarique et génération de CRP par stockage et traitements

	Char- donnay (Reg. II)	Chenin blanc (Reg. II)	Grenache (Reg. IV)	Ruby Cabernet (Reg. IV)	Semillon (Reg. IV)	Thompson Seedless (Reg. IV)
Juice, fully protected						
A. caftaric mg/l	191	100	201	67	90	64
% loss/wk (refrigerated)	1.5	2.7	—	—	10.6	3.8
CRP mg/l	0	0	0	0	0	0
Intact berry, refrigerated 0 °C in air (caftaric, no CRP, coumaric 0)						
% loss/wk from A	9.5	—	—	—	—	—
Juice, air exposed, SO₂ and ascorbic acid added as crushed						
caftaric mg/l	187	90	193	67	79	41
% loss from A	2	10	4	0	12	38
CRP mg/l	0	0	0	0	0	0
Juice, laboratory, unprotected						
B. caftaric mg/l	95	17	101	0	4	0
% of A	50	17	50	0	4	0
C. CRP mg/l	21	50	56	64	56	38
% of A	11	50	28	96	62	59
% disappeared [A - (B + C)]/A	39	33	22	4	34	5
Juice, winery, immediately pressed, 50 ppm SO₂						
D. caftaric mg/l	124	54	107	0	3	13
% of A	65	54	53	0	3	20
E. CRP mg/l	23	16	44	49	57	32
% of A	12	16	22	73	63	50
% disappeared [A - (D + E)]/A	23	30	25	27	31	30
Juice, racked after overnight cold settling						
caftaric mg/l	115	60	—	—	3	14
% change from D	- 7	+ 11	—	—	0	+ 8
CRP mg/l	23	17	—	—	56	31
% change from E	0	+ 6	—	—	- 2	- 3
Fermented 5 d with pomace						
F. caftaric mg/l	124	46	—	7	0	46
% change from D	0	- 15	—	+	- 100	+ 250
G. CRP	12	7	—	3	28	18
% change from E	- 48	- 56	—	- 94	- 51	- 44
% disappearance [A - (F + G)]/A	29	47	—	85	69	0

caftaric acid as a proportion of 320 nm absorbance from Californian wine as from an English wine (SINGLETON *et al.* 1978). This was true in spite of meticulous attention to details of the isolation procedure including use of a portion of the same lot of activated carbon used in England. We now know the Californian wines had less unmodified caftaric acid and thus the difficulty. Owing to shortage of winemaking equipment, the English wine was made by placing grapes, inoculum and SO₂ within a polyethylene bag, flushing the bag with CO₂ and then crushing the grapes by treading the bag. The same technique applied to Californian grapes gave wine with little CRP and more caftaric acid. When a large lot (400 kg) of Napa Gamay grapes was crushed with 900 g each of ascorbic acid and potassium metabisulfite in a strong stream of CO₂, the drained and settled juice retained 42 mg/l of caftaric acid and 68 % of that absorbed on charcoal was recovered in the eluate — a recovery better than the best (54 % overall, 61 % of adsorbed) obtained in England.

The CRP was considerably more resistant to extraction than caftaric acid and this at least partly explains the poor recovery of 320 nm absorbance from solvent extraction of ordinary musts and wines (Table 3). CRP was found in wines up to at least 2 years old. In some instances caftaric acid was also present and in others it was gone.

Table 3
Distribution coefficients (A₃₂₀ in solvent/A₃₂₀ aqueous)
Coefficients de distribution (A₃₂₀ en solvent/A₃₂₀ aqueux)

	EtOH/satd. (NH ₄) ₂ SO ₄	EtOAc
Coutaric acid	10.3	3.4
Caftaric acid	6.8	1.1
CRP	1.5	0.2
Wine (60 mg/l CRP, no caftaric)	1.0	0.1

The values previously reported in the literature for caftaric acid content cannot be evaluated correctly because the degree of oxygen exposure is unknown. The highest values reported here are higher than some previously reported for ripe grapes of the same varieties, but for other varieties they appear similar. Most of the previous reports indicated efforts to minimize oxidation, but perhaps not as complete as we found necessary. ONG and NAGEL (1978), for example, froze the berries in liquid nitrogen, thawed them in a microwave oven and crushed them with 1,000 ppm SO₂. Caftaric values obtained were high, but some oxidative modification appears possible. Other workers have been apparently less careful in some instances and any values obtained on ordinary winery musts do not represent accurately the original berry content of caftaric acid.

There are no data to indicate that the formation of CRP is deleterious. It must always have been a usual but unrecognized component of wines. Its recognition should, however, lead to useful new understanding as more is learned about it. Furthermore, the relative content of caftaric, CRP, and caffeic acid should serve as indicators of the original handling of the must and vinification. It is gratifying that the amounts of caftaric acid originally present in grapes and the sum of that remaining plus reaction products approach those estimated from gross evaluations of the phenol content by different means (SINGLETON 1982).

Summary

Grapes were crushed in the presence of high levels of the anti-oxidants SO_2 and ascorbic acid, in an atmosphere deprived of oxygen. Hydroxycinnamic derivatives, particularly caftaric acid in the grape juice were found in much higher amounts than when the usual processing methods were employed.

Partial oxidation of caftaric acid is not only very rapid and extensive during unprotected grape crushing and juice separation but gives a major reaction product that behaves as a single entity and survives through vinification. It gives a sharp HPLC peak and behaves reciprocally with caftaric, i.e., when caftaric acid in must remains high relative to the berry content the reaction product is low and *vice versa*. The product appeared identical in all grape varieties tested.

The reaction product is about one-sixth as extractable by immiscible solvents as is the parent caftaric acid. It can constitute a large part of the 320 nm absorbance of a wine and explains the variable results and poor yields in attempts to isolate caftaric acid from ordinary wine or juice.

Acknowledgements

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CAFTARIC AND COUTARIC ACIDS IN FRUIT OF *VITIS*

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Key Word Index—*Vitis*; Vitaceae; grape; chemotaxonomy; hydroxycinnamates; caftaric acid; coutaric acid; phenols.

Abstract—The contents of *trans* and *cis* caftaric and coutaric acids were determined by HPLC under conditions fully protecting against oxidation in berry juices from five non-*Vitis* species, 28 *Vitis* species, 20 white and 17 red *Vitis vinifera* cultivars, six *Vitis labrusca* cultivars and 11 other varieties. These components appear to be genetically determined to a considerable degree. Replicate samples of the same variety from different sources generally gave similar composition. The compounds were absent from or present only in trace amounts in *Vitis rotundifolia* and four (of five) non-*Vitis* species. Other *Vitis* species all contained them but the content ranged from 5 mg/l to 1350 mg/l of caftaric and from a trace to 340 mg/l of coutaric acid. Cultivars of *Vitis vinifera* did not differ appreciably whether white or red berries were involved, but ranged from 16 to 430 mg/l of *trans*-caftaric acid, averaging 145 mg/l. *Vitis labrusca* and its cultivars averaged notably higher and hybrids were generally intermediate with their parent species. The *cis* percentage increased, particularly for coutaric with the varieties arranged in order of decreasing caftaric concentration, but appeared to reflect environmental rather than genetic influences. The proportion of coutaric acid, however, appeared quite variable (3–33% of the caftaric content in the *vinifera* cultivars) by variety and was apparently genetically controlled.

INTRODUCTION

Considering 'fruit' in the farmer-consumer sense of the word, grapes are the most economically important fruit worldwide. The major phenols present in the readily expressed juice, and white wine made from it, of nearly all grape cultivars are the hydroxycinnamates [1]. Caftaric [monocaffeoyl-L-(+)-tartaric] acid is generally present at roughly 100 mg/l with the analogous *p*-coumaric (coutaric) and ferulic (fertaric acid) derivatives present at about 20 and 5 mg/l, respectively [2]. These compounds in grapes replace the chlorogenic analogs present in most other plants [1, 2]. Various processing and storage reactions such as browning depend upon the kinds and amounts of different phenols naturally present. Therefore, it is important to know the content of these compounds in cultivars and the genetic variation among species of *Vitis*.

This study also has more general interest in that chlorogenic acid analogs have had little use as chemotaxonomic markers and presumably could function similarly. Tartaric acid is rare as the major acid in plants except for grapes, but caffeic esters of tartaric acid have been reported for chicory, endive, lettuce and spinach [3–5]. Discovery that an immediate major product of phenolase oxidation in grape juice is *S*-glutathionylcaftaric acid emphasized the lability of these compounds [6, 7]. It also provided a technique for measuring whether oxidation had been completely prevented and cast doubt on the few previous related studies that might have involved some oxidative losses.

This study was designed to determine the content of caftaric and coutaric acids and their glutathione thioether adduct, if any, under conditions designed to preclude oxidative changes during sampling. All commercial grape cultivars planted to the extent of 1500 or more acres in California, several cultivars such as Concord important elsewhere in the U.S., and as many *Vitis* species as possible

were sampled. Objectives included estimation of the content of *trans*- and *cis*-forms and the ratios between caftaric and coutaric acids with respect to cultivar groups and species.

RESULTS AND DISCUSSION

The addition of sulphite to suppress polyphenolase action, ascorbic acid to reduce any quinone formed, and a CO₂-filled glove box to exclude oxygen as the berries were crushed were successful in all cases in preventing the formation of *S*-glutathionylcaftaric acid [7]. It was completely absent in most samples. Traces too low to quantify (< 0.5 mg/l) were found in the samples for *Vitis shuttleworthii*, *V. berlandieri*, *V. champini*, Black Corinth, one Pinot noir and Gewurztraminer. These traces are attributed to berry damage at the berry-pedicle junction during harvesting. These were generally soft or overripe samples with either very small berries or tight clusters.

An effort was made to harvest the berry samples near 20° Brix to minimize any effect from ripeness variation. This was not always possible because some samples either failed to reach the desired level or were already past it when sampled. However, studies separately reported [8] indicate that grapes tend to maintain a relatively constant content of hydroxycinnamates. The level characteristic for a variety only fell appreciably during ripening if berry enlargement was considerable and content high so that synthesis failed to keep up. It may rise as ripe berries shrivel. Therefore, it is believed that the relatively small ripeness differences near commercial ripeness are not a serious complication.

It is also believed that vineyard location and vintage year (weather) have relatively little influence compared to the genetic characteristics of the mother vine, but they do not appear to be without influence. Clonal differences

within varieties may be factors especially in widely separated vineyards. A study [10] of White Riesling wines showed considerable variation among samples from different sources and vintages in their caftaric acid content yet averaging the same for California and Washington samples (123 mg/l). Alsatian samples averaged significantly lower, but again were fairly constant within their group over three vintages. Since these were wine samples, losses by conversion to glutathionylcaftaric undoubtedly occurred, but were not determined. A White Riesling wine prepared commercially had 66 mg/l of caftaric and, in contrast to wines from other varieties, no glutathionylcaftaric acid [7]. This will be considered further below, but it appears that the grape selection has the greatest influence on the juice content of hydroxycinnamates. The data of this report were all on 1984 vintage fruit harvested from two vineyards under the same management and about 60 km apart.

Most of the results are shown in Tables 1–4 arranged in decreasing order of caftaric acid content within each table. Not shown are the data obtained on fruit of *Ampelopsis brevipedunculata elegans*, *A. aconitifolia glabra*, *Zizyphus jujuba* (Rhamnaceae) and *Parthenocissus quinquefolia* (Vitaceae) all of which showed none or perhaps traces (in different chromatographic patterns) of *trans*-caftaric acid or its companions. *Parthenocissus tricuspidata* had 25.6 mg/l of *trans*-caftaric, 12.8 mg/l of *trans*-coutaric acid and traces of the respective *cis*-isomers. *Vitis rotundifolia* in three examples had none or traces of *trans*-caftaric acid. All other species and cultivars of *Vitis* (Tables 1–4) had at least 5 mg/l and up to 1337 mg/l of *trans*-caftaric acid. It seems significant that *V. rotundifolia* is considered, by some authorities, a separate genus or subgenus *Muscadinia* as opposed to *Euvitis*, the 'true' grapes. It appears that caftaric acid and its relatives are universal and characteristic within the genus *Vitis* with the exception of *V. rotundifolia*. As far as these data allow conclusions, caftaric acid appears of limited occurrence outside this genus. It is noteworthy that *V. rotundifolia* crossed with *V. vinifera* produced hybrids (two examples Table 4) with only low levels of *trans*-caftaric acid but an anomalously high apparent level of coutaric acid in one of them.

If the percentage of either acid that is *cis* is examined, in all four tables, it is seen to generally increase, particularly for coutaric acid, as the caftaric content decreases. For the data in Table 3, for example, the coefficient of linear correlation between *trans*-caftaric acid content and the percentage of the coutaric that was *cis* on a linear regression basis was -0.73 , and for the *cis* percentage of caftaric was -0.62 . The mean proportion of the caftaric acid that was *cis* was about 2% and of the coutaric was about 18%. These proportions of *cis*-forms seem more a function of environmental conditions than of genetic source and have low but about equal variability between replicates of the same variety as among varieties.

The percentage of *trans*-coutaric acid concentration relative to *trans*-caftaric acid concentration is evidently predominately influenced by genetics, but still has a linear correlation coefficient with *trans*-caftaric acid content of -0.42 . Note in Tables 1–3 that replicate samples of the same variety or species from different vineyard sources were quite similar in the percent of *trans*-coutaric relative to *trans*-caftaric acid. *Vitis shuttleworthii*, St. Emillion, and Ribier seem notably low in the proportion of coutaric acid and *V. cordifolia*, *V. cinerea*, *V. solonis*, *V. aestivalis*,

Emerald Riesling, Ruby Seedless, Emperor, Carignane, Ruby Cabernet, Rubired, and Salvador notably high. The one selection (but not the other) of a *V. vinifera* $\times$ *V. rotundifolia* cross was in a class by itself having four times as much apparent coutaric acid content as caftaric acid. Remembering that the identification and quantitation here depends on HPLC retention and peak area characteristics of known caftaric acid, it is possible that this result involves contamination of the identified peak with an unknown substance absorbing strongly at 320 nm. The chromatograms in this instance gave a large, symmetrical, well separated peak in the proper position, but it was not possible to study it further. An additional large, slower peak was unusual in this fruit indicating also an unusual amount of fertaric or another unidentified component. Note (Table 1) that one selection of *V. rotundifolia* anomalously appeared to have 4 ppm of *cis*-coutaric but no *trans*-coutaric acid, suggesting misidentification of that peak.

The caftaric acid contents reported are means of duplicate analyses of separate berry samples. For the 26 examples where 40 berries were used for each duplicate, the average deviation of *trans*-caftaric acid was $\pm 1.5\%$ from the mean of the two, i.e. quite close agreement combining both the berry and analytical variation. Three separate Grenache sources (Table 3) had a relatively high and constant *trans*-caftaric acid content, 261–290 mg/l. Literature values for this variety for different years and widely separated vineyards invariably indicate this variety to be high relative to others and fairly close to these values [2, 6, 9]. Other comparisons with the literature could be made and agree roughly, but owing to uncertainty about oxidative change in the literature data, they were not considered useful. Note that in Tables 1–3 the nine examples of more than one set of samples representing different vines from the same species or cultivar agree well for *V. monticola*, Chardonnay, Grenache, Pinot noir, Cabernet Sauvignon and Ruby Cabernet not only in *trans*-caftaric content, but also in *trans*-coutaric acid content and other details. The sets for Semillon and French Colombard did not agree well but there was a large ripeness difference, and Carignane also did not but there was a large berry size difference and the amounts per berry were relatively close. Further comparisons among the eight varieties for which values were available from 1982 and 1983 vintages [6] gave an average variation from the mean of $\pm 20\%$ for single varieties and more than double that among varieties. These data indicate the juice content of these compounds is primarily determined by the genetic make-up of the particular species or cultivar.

Several points of interest become apparent in close study of the data. Palomino is highest in caftaric acid content especially on a per berry basis and is noted for making quality sherry wines. White grapes noted for consumption as table grapes (Malaga, Perlette, Thompson Seedless, Calmeria) are generally low in caftaric acid content (Table 2). With the notable exception of Ribier which had a very high juice concentration and the most caftaric acid per berry of any species or variety, the red table varieties tended to follow the same pattern (Flame Tokay, Ruby Seedless, Emperor; Table 3), although Cardinal was intermediate.

The red *vinifera* cultivars (Table 3) averaged slightly higher in both caftaric and coutaric acid content than did the white ones (Table 2), but they overlapped for all but the extreme examples of Carnelian and Calmeria. With

Table 1. Caftaric and coumaric acid contents of juice of berries of *Vitis* species

Vitis species	Juice Brix	Berry wt (g)	Caftaric (Ca) acid				Coumaric (Cu) acid				%		µg/berry	
			(mg/l)		%		(mg/l)		%		t-Cu t-Ca		t-Ca	
			trans	cis	% cis	% cis	trans	cis	% cis	% cis	t-Cu	t-Ca	t-Ca	t-Ca
<i>aestivalis</i>	25.2	0.55	1337	16	1.2	1.2	326	16	4.5	4.5	24	618	151	—
<i>monticola</i>	19.8	0.57	1155	4	0.3	0.3	59	3	5.2	5.2	5	563	29	—
<i>monticola</i>	21.8	0.57	1010	6	0.6	0.6	43	2	4.1	4.1	4	496	21	—
<i>rufotomentosa</i>	15.1	0.21	772	2	0.2	0.2	30	2	6.0	6.0	4	127	5	—
<i>simpsonii</i> (pixiala)	23.3	0.34	664	6	1.0	1.0	21	tr	—	—	3	184	6	—
<i>arizonica</i>	27.4	0.20	484	0.2	0.1	0.1	34	5	15.9	15.9	7	67	5	—
<i>labrusca</i>	19.1	2.18	462	6	1.3	1.3	43	3	7.0	7.0	9	918	85	—
<i>smiliana</i>	7.2	0.42	443	2	0.4	0.4	24	2	7.5	7.5	5	163	9	—
<i>trelasei</i>	15.8	0.61	405	2	0.4	0.4	36	6	17.3	17.3	9	217	19	—
<i>candicans</i>	23.2	0.41	403	4	1.1	1.1	66	19	28.1	28.1	16	136	22	—
<i>linceumii</i>	16.6	1.60	246	4	1.8	1.8	35	3	9.4	9.4	14	362	52	—
Afghanistan source	21.6	0.44	220	3	1.2	1.2	14	tr	—	—	6	82	5	—
<i>girdiana</i>	16.1	0.34	207	1	0.4	0.4	30	5	17.6	17.6	14	57	8	—
<i>shuttleworthii</i>	20.1	1.17	185	2	1.1	1.1	3	tr	—	—	2	193	3	—
<i>amurensis</i>	17.8	0.57	128	2	1.7	1.7	22	5	20.8	20.8	17	61	11	—
<i>californica</i>	22.5	1.46	122	3	2.8	2.8	11	1	12.8	12.8	9	159	4	—
<i>berlandieri</i>	14.8	0.35	89	1	1.1	1.1	13	tr	—	—	14	26	4	—
<i>novae-angliae</i>	24.7	1.38	71	4	5.0	5.0	13	2	15.8	15.8	18	87	16	—
<i>doaniana</i>	18.6	1.42	57	0.5	0.8	0.8	7	1	16.9	16.9	12	73	9	—
<i>cinerea</i>	16.6	0.27	53	tr	—	—	23	3	11.7	11.7	43	12	5	—
<i>slavii</i>	22.8	0.57	48	tr	—	—	5	2	33.3	33.3	10	24	2	—
<i>longii</i>	21.4	0.69	43	tr	—	—	8	2	29.6	29.6	19	26	5	—
<i>champini</i>	20.4	0.74	43	tr	—	—	6	7	112.9	112.9	14	28	4	—
<i>riparia</i>	18.9	0.29	41	2	3.9	3.9	6	tr	—	—	15	10	1	—
<i>rupestris</i>	16.3	0.29	40	tr	—	—	8	1	14.3	14.3	19	10	2	—
<i>solonis</i>	19.6	0.79	17	tr	—	—	6	tr	—	—	32	12	4	—
<i>cordifolia</i>	22.6	0.20	5	tr	—	—	6	2	20.7	20.7	135	1	1	—
<i>rotundifolia</i> (Thomas)	15.1	4.47	0	0	0	0	0	0	0	0	0	0	0	—
<i>rotundifolia</i> (Creek)	16.1	3.18	tr	0	0	0	0	0	0	0	0	tr	0	—
<i>rotundifolia</i>	18.1	0.95	1.5	tr	—	—	0	4	—	—	0	1.3	0	—
Mean	19.3	0.91	292	—	1.3	1.3	—	—	17.8	17.8	16.6	—	—	—

Table 2. Caftaric and coumaric acid contents of juice of commercially important white varieties of *Vitis vinifera*

Cultivar	Juice Brix	Berry wt (g)	Caftaric (Ca) acid			Coumaric (Cu) acid			%		$\mu\text{g/berry}$	
			trans (mg/l)	cis (mg/l)	% cis	trans (mg/l)	cis (mg/l)	% cis	t-Cu t-Ca	t-Ca	t-Ca	
Palomino	20.2	2.42	295	4	1.5	42	5	9.9	14.2	648	92	
Pinot blanc	20.8	1.44	266	3	1.2	18	1	6.8	6.7	255	17	
Semillon (D)*	11.3	1.13	197	2	1.0	11	tr	—	5.5	206	11	
Semillon (N)*	21.2	2.17	98	1	1.3	4	tr	—	4.1	193	8	
Rkatsiteli	20.8	2.05	181	4	2.4	19	5	20.2	10.3	337	35	
St. Emilion	20.6	1.79	179	5	2.9	6	1	18.8	3.1	290	9	
Emerald Riesling	21.3	1.84	154	2	1.5	47	6	11.8	30.7	255	78	
Muscat blanc	22.4	2.11	143	4	2.6	24	6	19.3	16.6	272	45	
Muscat Alexandria	17.0	3.57	141	3	1.9	6	2	20.5	4.4	468	21	
French Colombard	8.7	1.15	142	1	0.7	18	3	15.0	12.8	153	20	
French Colombard	19.8	1.79	97	2	2.5	15	4	21.5	15.5	157	24	
Chardonnay (D)	22.1	1.14	135	1	0.4	15	4	18.7	11.3	136	15	
Chardonnay (N)	21.0	1.55	122	3	2.6	12	3	19.4	9.5	170	16	
Burger	19.5	2.68	119	2	1.6	4	tr	—	3.6	291	11	
Chenin blanc	20.6	1.94	110	2	2.2	11	3	20.6	9.8	193	19	
White Riesling	20.7	1.91	109	3	2.5	8	3	25.2	7.6	188	14	
Sauvignon blanc	22.4	1.63	97	2	2.0	12	3	18.7	12.6	140	18	
Grey Riesling	23.9	1.59	81	2	1.9	15	4	20.7	17.9	115	21	
Malaga	19.0	3.89	61	2	4.0	11	2	19.1	17.5	217	38	
Perlette	19.0	2.65	59	3	4.5	6	2	29.3	9.8	144	14	
Thompson Seedless	21.5	1.34	56	2	3.3	3	1	18.8	4.6	52	3	
Gewurztraminer	20.1	1.27	54	1	1.8	3	1	23.8	5.9	62	4	
Calmeria	18.8	4.64	16	0	—	tr	tr	—	—	68	tr	
Mean	19.7	2.07	127	2.4	2.1	14	3.0	18.8	10.6	218	24	

* D = Davis vineyard (warmer, earlier), if not designated this was the source; N = Napa Valley vineyard (cooler, later).

Table 3. Caftaric and coutaric acid contents of juice of commercially important red varieties of *Vitis vinifera*

Cultivar	Juice Brix	Berry wt (g)	Caftaric (Ca) acid				Coutaric (Cu) acid				%		µg/berry	
			trans (mg/l)	cis (mg/l)	% cis	trans (mg/l)	cis (mg/l)	% cis	trans (mg/l)	cis (mg/l)	t-Cu t-Ca	t-Cu t-Ca	t-Ca	t-Ca
Carnelian	20.7	1.63	430	5	1.1	39	3	6.7	9.0	630	57	3.2	1523	49
Ribier	19.7	5.63	294	4	1.3	9	tr	—	3.2	1523	49	7.0	327	23
Grenache (D)*	18.9	1.26	290	4	1.3	20	1	6.5	7.8	291	23	6.7	379	25
Grenache (D)	20.2	1.22	267	2	0.7	21	2	9.6	7.0	45	7	16.1	365	59
Grenache (N)*	21.0	1.62	261	3	1.2	17	1	7.0	18.5	274	51	6.4	328	21
Black Corinth	23.4	0.22	221	2	0.9	31	4	10.2	5.6	92	5	5.7	150	9
Mission	20.1	2.23	216	3	1.5	35	4	9.4	7.4	129	10	8.0	110	9
Petite Sirah	21.8	1.62	188	3	1.4	35	5	13.4	6.9	795	55	26.4	152	40
Zinfandel	21.4	2.13	171	4	2.0	11	2	12.7	25.8	112	29	23.1	142	33
Pinot noir (D)	21.7	0.66	160	2	1.3	9	3	26.2	19.3	232	31	6.6	408	27
Pinot noir (N)	19.3	1.20	139	2	1.2	8	1	6.9	20.2	71	7	9.5	71	7
Cabernet Sauvignon (N)	23.2	1.05	140	6	3.9	10	4	26.8	33.3	132	44	8.8	107	9
Cabernet Sauvignon (D)	22.0	1.12	110	1	1.2	9	2	17.8	29.0	153	31	13.4	298	28
Cardinal	17.6	6.12	140	4	2.8	10	tr	—	6.9	795	55	26.4	152	40
Carignane (D)	20.6	1.54	109	2	1.7	29	5	15.3	26.4	152	40	25.8	112	29
Carignane (N)	19.6	2.16	56	1	2.4	14	3	18.2	23.1	142	33	6.6	408	27
Ruby Cabernet (D)	16.6	1.44	108	2	2.0	25	8	23.3	20.2	71	7	9.5	71	7
Ruby Cabernet (N)	24.2	1.45	103	2	2.3	24	6	19.3	33.3	132	44	8.8	107	9
Flame Tokay	20.2	4.77	93	4	4.5	6	2	27.9	29.0	153	31	13.4	298	28
Merlot	22.4	1.02	79	2	2.1	8	2	20.2	9.5	71	7	9.5	71	7
Ruby Seedless	21.4	2.35	61	2	3.0	20	8	28.4	33.3	132	44	8.8	107	9
Gamay (Napa)	19.5	2.31	57	2	2.9	5	2	27.5	29.0	153	31	13.4	298	28
Emperor	17.4	3.44	48	2	4.2	10	4	20.4	29.0	153	31	13.4	298	28
Mean	20.6	2.10	163	2.8	2.0	18	3.4	16.8	13.4	298	28	13.4	298	28

*D = Davis vineyard (warmer, earlier), if not designated this was the source; N = Napa Valley vineyard (cooler, later).

Table 4. Caftaric and coutaric acid contents of red juice or non-*vinifera* cultivars

Cultivar	Juice Brix	Berry wt (g)	Caftaric (Ca) acid			Coutaric (Cu) acid			%	µg/berry			
			trans (mg/l)	cis (mg/l)	% cis	trans (mg/l)	cis (mg/l)	% cis		t-Ca	t-Cu t-Ca		
Red Juice varieties													
Alicante Bouschet	16.6	2.21	238	2	1.0	30	4	12.6	12.5	484	61	484	61
Salvador	20.0	1.15	171	2	1.4	44	5	9.7	25.8	175	45	175	45
Saperavi	21.8	1.52	168	3	1.6	28	5	14.2	16.9	229	39	229	39
Rubired	22.2	1.40	87	2	1.1	31	7	18.5	35.2	109	39	109	39
Labrusca varieties													
Catawba	17.6	2.10	322	4	1.2	36	4	9.5	11.3	620	70	620	70
Niagara	15.1	3.08	280	5	1.8	41	4	9.5	14.6	807	118	807	118
Concord	17.0	2.62	223	2	1.1	12	tr	—	5.6	539	30	539	30
Isabella	16.0	2.36	155	1	0.9	21	2	9.2	13.4	338	45	338	45
Niabell	14.9	5.30	131	2	1.8	10	tr	—	7.3	653	48	653	48
Delaware	20.0	1.61	104	2	2.0	17	1	4.4	16.6	151	25	151	25
Hybrid varieties													
Vidal 256	19.0	1.22	107	1	0.6	12	2	12.7	11.0	117	13	117	13
DeChaunac (Seibel 9549)	18.9	1.36	94	2	1.9	15	2	13.6	16.1	115	19	115	19
Foch	22.1	1.10	62	2	2.7	4	tr	—	7.1	61	4	61	4
Aurore (Seibel 5279)	17.6	1.56	56	2	4.3	8	2	23.1	14.8	80	12	80	12
Baco noir (No. 1)	20.6	1.02	47	tr	—	3	tr	—	7.3	42	3	42	3
<i>vinifera</i> × <i>rotundifolia</i>	24.0	0.43	14	tr	—	2	1	26.7	16.3	5	1	5	1
<i>vinifera</i> × <i>rotundifolia</i>	25.4	0.36	16	tr	—	66	10	13.2	422.3	5	20	5	20

respect to relative coumaric acid content in these varieties, Emerald Riesling, Ruby Seedless, Emperor, Carignane, and Ruby Cabernet are high and St. Emilion and Ribier low. The wild grape species tend to be higher in caftaric and coumaric acid concentration (Table 1) compared to the European wine grape (Tables 2 and 3), but 18 of the 27 species had less than 300 mg/l of caftaric acid. Because the wild species mostly have small berries, the amounts per berry are generally smaller than the cultivars. *V. aestivalis* was outstandingly high in both caftaric and coumaric acids with *V. monticola* very high in caftaric concentration. *V. labrusca* was highest among those North American species most used in developing fruited varieties and this is reflected in varieties derived from it, notably Catawba, Niagara and Concord (Table 4).

The other varieties most used in breeding work and rootstocks *V. riparia* and *V. rupestris* (Table 1) are low as are the direct producer hybrids (Table 4). Relatively high proportions (but not concentration) of *trans*-coumaric acid occurred in *V. cinerea* and *V. cordifolia* (Table 1), and one of the *vinifera* × *rotundifolia* crosses (Table 4) as already mentioned. The red-juice grapes covered the range noted in other varieties (Table 4) and do not seem as a group to be unusual in their hydroxycinnamate content even though they are in anthocyanin content.

The chromatograms for the *vinifera* varieties were relatively similar, whereas those from non-*vinifera* varieties and species often produced unusual patterns and large unknown peaks. For example, Isabella and *V. monticola* gave two sizeable long-retained peaks not noted in others. *V. solonis* and Baco noir appeared to have other substances with similar retentions including apparently relatively high ferulic acid content. Oxidation of Aurore juice gave in addition to glutathionylcaftaric acid considerable of what appears to be cysteinylcaftaric acid.

EXPERIMENTAL

Fruit sources. The designations carried in the records and vine markings of the variety and species collections of the University of California, Davis, were used. After a field estimation of Brix, the chosen vines were sampled (usually 7–8 AM) so as to represent the total fruit present (either all available clusters or ones selected randomly over the vine) with clusters or cluster parts sufficient to yield at least 30 berries or 40 g. The individual berries were snipped with small scissors so as to remove the pedicel with the torus without breaking the berry skin. Any berries seen to be damaged were discarded.

The berries were counted, weighed and placed in a glove box under a flowing CO₂ atmosphere. There they were mixed with

solid ascorbic acid and potassium metabisulphite (ca 200 mg each) and then wrapped in cheesecloth and pressed in a small, levered hand-press as uniformly as possible. The juice was filtered (prefilter with Celite followed by 0.45 µm membrane) and chromatographed on a C₁₈ reversed phase column with pH 2.6 16% aq. MeOH essentially as described before [6, 7]. Peak areas were converted to amounts by using the peak area per unit mass determined under the same conditions with pure *trans*-caftaric acid isolated from grapes. The *trans*-coumaric acid content was estimated from the same response factor. The amounts determined by the same method for the *cis*-forms were multiplied by 1.58 which is the relative absorbance difference, thus providing a better estimate of the true content.

Juice Brix was determined by refractometry on a separate sample. The percentage *cis* was calculated as the proportion of the sum of the two forms determined for either caftaric or for coumaric acids. The weight of each substance per berry was calculated from the berry weight minus an estimated standard weight of 40 mg for seeds in each seeded berry, divided by the density equivalent to the juice Brix, times the concn of each substance. The seed number increased to near four per berry for the small wild berries, but the average seed size dropped, so a standard estimate of seed amount per berry was deemed adequate for the present purposes. The amounts per berry were probably overestimated especially on smaller berries since no correction was made for the berry skin, which has a lower concentration per unit weight of hydroxycinnamates.

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