UNIVERSIDAD DE CÓRDOBA

Programa de doctorado

Biociencias y Ciencias Agroalimentarias

Título de la tesis

Cinética del proceso de deshidratación de frutos rojos y elaboración de bebidas fermentadas. Cambios en el color y compuestos bioactivos durante el procesado.

Kinetics of drying process of red fruits and elaboration of fermented beverages. Color and bioactive compounds changes during processing.

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Córdoba, a 13 de Diciembre de 2019

TITULO: *CINÉTICA DEL PROCESO DE DESHIDRATACIÓN DE FRUTOS ROJOS Y ELABORACIÓN DE BEBIDAS FERMENTADAS. CAMBIOS EN EL COLOR Y COMPUESTOS BIOACTIVOS DURANTE EL PROCESADO*

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TÍTULO DE LA TESIS: Cinética del proceso de deshidratación de frutos rojos y elaboración de bebidas fermentadas. Cambios en el color y compuestos bioactivos durante el procesado.

DOCTORANDO: Juan Martín Gómez

INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS

La citada Memoria de Tesis Doctoral se ha realizado utilizando una metodología adecuada y haciendo uso de las técnicas instrumentales idóneas para culminar los objetivos previstos en el Plan de Investigación. Asimismo, el licenciado Martín Gómez ha cumplido con todas las tareas encomendadas para la realización del plan de investigación previsto, desde el diseño de los experimentos hasta la redacción de los artículos científicos que conforman su Tesis Doctoral. Desde su incorporación al Programa de Doctorado ha estado muy implicado en el proyecto de Tesis, tomando decisiones sobre su proyecto y adaptándose a las complicaciones generadas de él y mostrando en todo momento una alta dedicación e interés por el tema de trabajo, superando con creces los niveles de calidad científica exigibles para la obtención del Grado de Doctor. Durante su formación ha realizado varios cursos de formación para complementar su labor investigadora. Como consecuencia de su trabajo, ha publicado varios artículos derivados directamente de su trabajo, y que conforma la Tesis, en revistas internacionales de alto impacto indexadas en el Journal Citation Report (JCR) y ha presentado numerosas comunicaciones en congresos, tanto nacionales como internacionales, algunas de ellas con capítulos de libros publicados. Además, debido a la colaboración en las tareas investigadoras del Grupo de Investigación posee otros artículos en revistas internacionales indexadas también en JCR. Esta tesis se considera que reúne las condiciones académicas exigidas por la legislación vigente para aspirar al Grado de Doctor por la Universidad de Córdoba.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 12 de diciembre de 2019

Firma de las directoras

Fdo.: Julieta Mérida García

Fdo.: María Pérez Serratosa

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1. FRUTOS ROJOS

Los frutos rojos o frutos del bosque, conocidos así por el color llamativo de su piel o su interior, son pequeños frutos comestibles tipo baya, carnosos, jugosos, dulces o ácidos. Hay numerosos frutos de este tipo, pero entre los más cultivados nos podemos encontrar con la uva, el arándano azul, el arándano rojo, la cereza, la fresa, la grosella, la zarzamora y la ciruela (Figura 1).

Figura 1. Tipos de frutos rojos.

Tradicionalmente no eran cultivados si no que crecían silvestres en arbustos. Pero actualmente, su cultivo está en pleno auge, puesto que los frutos rojos son conocidos por ser ricos en nutrientes: como azúcares, proteínas, fibra y minerales; pero además, también presentan un alto contenido en compuestos bioactivos (Y. Sun *et al.*, 2019): como los compuestos fenólicos (ácidos fenólicos, antocianos, flavonoles,…) o vitaminas como la vitamina C. Estos compuestos aportan a las bayas numerosas propiedades beneficiosas para la salud, por ejemplo, anticancerígenas, antidiabéticas, antiinflamatorias, antimicrobianas y antioxidantes (Baby *et al.*, 2018; Skrovankova *et al.*, 2015).

1.1. Uva. Origen, cultivo y clasificación

La vid es de la familia V*itaceae* del género *Vitis*. La vid silvestre de la especie *Sylvestris* es originaria de Asia occidental y su origen se establece hace 65 millones de años (Bergamini *et al.*, 2019; This *et al.*, 2006). Se han encontrado pipas carbonizadas de uva (Figura 2) de la prehistoria en numerosas zonas de

Europa, como Grecia, Yugoslavia, Italia, Suiza, Alemania y Francia (Valamoti, 2014; Zohary and Spiegel-Roy, 1975).

Figura 2. Semillas de uva carbonizadas halladas en Dikili Tash.

La uva es la fruta más cultivada del mundo (Abdel-Kawi *et al.*, 2016), siendo el mayor productor el continente europeo. Como se puede ver en los datos estadísticos obtenidos de *Food and Agriculture Organization of the United Nationes* (FAO, 2019), el continente europeo sufre una pérdida de producción entorno al año 1982, siendo el continente asiático y americano los que experimentan un aumento en su producción. Desde el punto de vista global, se percibe esta pérdida de producción en Europa, sin embargo la producción actual es una de las más altas registradas en los últimos años.

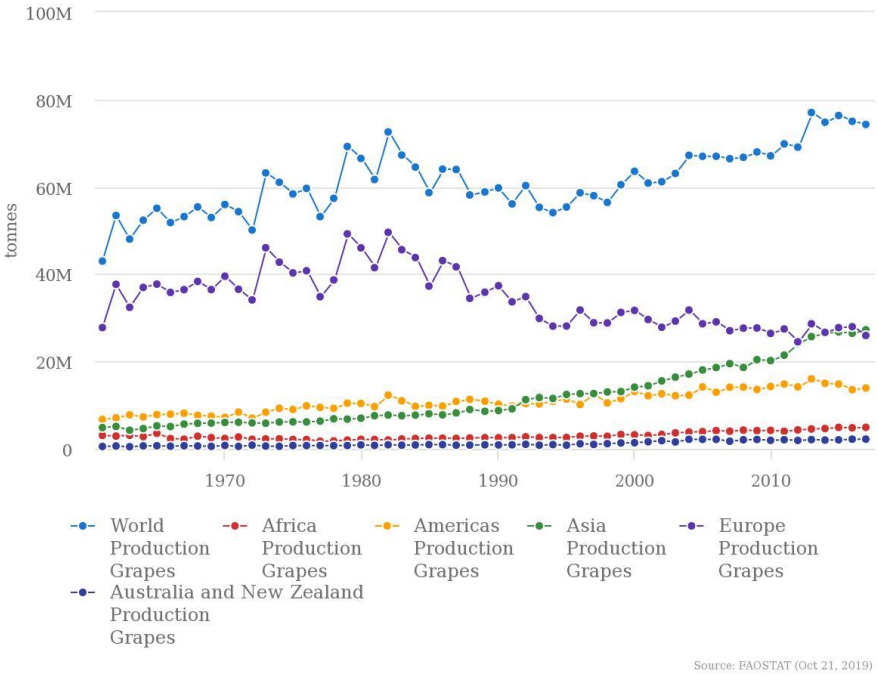


Figure 3. Producción de uva (Tn) en los 5 continentes, desde 1961 hasta 2017.

La especie *Vitis vinífera* es la más cultivada en todo el mundo, siendo la única especie de *Vitis* que ha alcanzado un interés económico a lo largo del tiempo (Ferreira *et al.*, 2018) por la calidad de sus frutos y su destino a consumo directo, vinificación o otros productos derivados de los mismos. Esta especie de vid es la única que se utiliza en el mundo en la industria del vino (This *et al.*, 2006), aunque podemos encontrar otras especies como *Vitis rupertris* norteamericana, *Vitis riparia* o *Vitis berlandieri* que se utilizan como portainjertos puesto que son resistentes a la filoxera, un insecto capaz de destruir grandes zonas de viñedos en muy poco tiempo.

Actualmente, según la base de datos de la Organización Internacional de la Viña y el Vino (OIV) se cuenta con unas 8111 variedades de uvas diferentes en todo el mundo. Estas variedades se pueden clasificar atendiendo a diferentes factores como su origen, el destino de la uva, el color de la piel, la forma de la baya, las denominaciones de origen, …

Según el destino habitual de la uva obtenida se pueden clasificar, como se refleja en el Reglamento de la Comunidad Europea nº 2389/89 del Consejo, de 24 de julio de 1989, relativo a las normas generales referentes a la clasificación de las variedades de vid.

- Variedad de uva de vinificación: una variedad de vid cultivada, de forma habitual, para la producción de uva destinada a la elaboración de vinos para el consumo humano directo.
- Variedad de uva de mesa: una variedad de vid autorizada en el marco de las normas comunes de calidad para las uvas de mesa, adoptadas en aplicación del Reglamento (CEE) Nº 1035/72 del Consejo, de 18 de mayo de 1972, por el que se establece la organización común de mercados en el sector de las frutas y hortalizas, modificado en último lugar por el Reglamento (CEE) Nº 1119/82 y que se cultive, de forma habitual, para la producción de uva destinada al consumo en fresco.
- Variedad de uva para un destino particular: una variedad de vid cultivada normalmente para otros destinos que los mencionados anteriormente:
  - elaboración de aguardiente de vino.
  - elaboración de zumo de uva.
  - producción de uva destinada normalmente a la industria conservera.
  - producción de uvas para secar.

- Variedad de portainjerto: una variedad de vid cultivada para la producción de material de multiplicación vegetativa de la vid y de la que se obtenga la parte subterránea de la planta.

Otra forma de clasificarlas es por el color de su piel (Fernández-López *et al.*, 1997), según el código 225 de la lista de descriptores de la OIV puede ser en los siguiente grupos: Verde-amarilla, rosa, roja, gris, roja-violeta oscura o azul-negra. Los datos recogidos en la base de datos de la OIV demuestran que en España existen 154 variedades de uva distintas, las cuales solo se pueden clasificar en verde-amarilla y en azul-negra.

Tabla 1. Principales variedades de uva cultivadas en España

Verde-amarilla		**Azul-negra**	
Variedad	Producción (ha)	Variedad	Producción (ha)
Airen	215484.14	Tempranillo	201051.39
Macabeo	44906.72	Bobal	61524.21
Cayetana blanca	39919.12	Garnacha	57907.35
Verdejo	19058.77	Monastrell	43049.18
Palomino	11283.72	Garnacha tintorera	22572.20
Moscatel de Alejandría	10318.04	Cabernet sauvicnon	20535.37
Xarello	9604.65	Syrah	20155.11
Palomino fino	8977.68	Merlot	13043.62
Pedro Ximenez	8960.34	Mencia	8330.63
Parellada	8230.77	Macuela	5644.42
Chardonnay	7674.11	Tinto de la pampa blanca	4718.95
Chelva	5729.97	Prieto picudo	4300.12
Albariño	5722.31		
Sauvignon blanco	5086.56		
Alarije	5010.75		
Beba	4470.72		
Zalema	4118.90		

El viñedo en España abarca 941154.86 ha cultivadas, según la encuesta sobre viñedo del 2015 del Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente del Gobierno de España. De las cuales 440153.66 ha están cultivadas con uva verde-amarilla y 484396.07 ha con uva azul-negra. Las variedades más cultivadas están reflejadas en la tabla 1, donde destacan las variedades Airen y Tempranillo.

1.2. Arándano azul. Origen, cultivo y clasificación

Los arándanos pertenecen a la familia *Ericaceae*, subfamilia *Vaccinoideae* y del género *Vaccinium*, su origen es desconocido aunque se cree que su desarrollo y diferenciación se remonta al período cretácico, hace más de 100 millones de años (Routray and Orsat, 2011), y que son originarios de Estados Unidos y Europa (Reque *et al.*, 2014; Santos *et al.*, 2016). Estas bayas silvestres han sido cosechadas desde hace cientos de años por nativos norteamericanos antes de su colonización (Figura 4). Los colonos europeos apreciaron estas bayas y las integraron en su alimentación, incluyendo por ejemplo la salsa de arándanos en recetas que se usan actualmente en el día de acción de gracias.

Figura 4. Nativas norteamericanas en la elaboración de un alimento basado en arándanos y carne.

A pesar de que antiguamente se consideraran bayas silvestres, los arándanos actualmente se cultivan en todo el mundo, como se puede ver en la figura 5 que resume los datos estadísticos recogidos por la FAO (FAO, 2019). Con estos datos se puede decir que la producción de arándanos en el mundo está en crecimiento exponencial desde los años 70, siendo claramente el continente americano el mayor productor de todos, seguido del continente europeo.

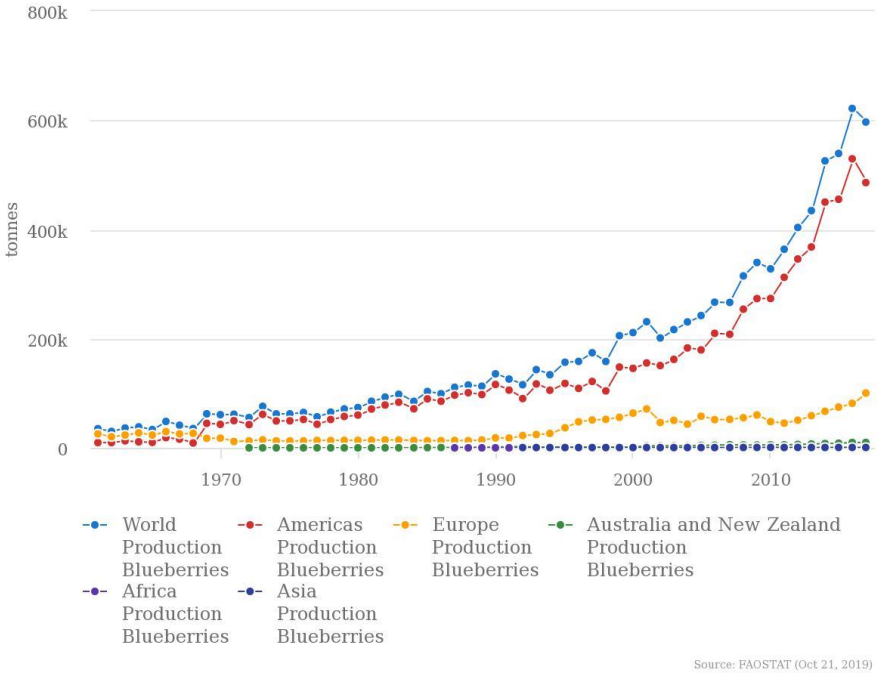


Figura 5. Producción de arándanos (Tn) en los 5 continentes, desde 1961 hasta 2017.

Los arándanos del género *Vaccinium*, presentan varias especies cultivadas, entre las que destacan por su cultivo *corymbosum*, *augustifolium* y *ashei* (Routray and Orsat, 2011). Los arándanos comprenden entre 400 y 500 especies de arbustos en todo el mundo (Tailor *et al.*, 2017), estos se pueden clasificar atendiendo a diferentes factores.

Según el tamaño del arbusto, se pueden clasificar como "*highbush"*o altos, a aquellos cuya altura esté comprendida entre 1.5 y 7 m; y *"lowbush"* o bajos , para los que tienen una altura inferior a 1 m.

Según las horas de frío que necesiten se pueden denominar *"northern"* o *"southern"*. Las que necesitan más de 800 horas de frío son las "*northern highbush*" o gigantes del norte representadas por la especie V*accinium corymbosum*, o las "*lowbush"* representadas por la especie V*accinium angustifolium*; las que precisan un rango de horas de frío medio comprendido entre 400 y 600 horas, como la especie V*accinium ashei* también conocida como "*rabbiteye"* u ojo de conejo; y, por último, aquellas que requieren menos de 400 horas de frío conocidas como "*southern highbush"* o gigantes del sur, que están representadas por especies cruzadas entre *Vaccinium corymbosum, Vaccinium ashei* y *Vaccinium darrowi.*

Según su época de maduración, se clasifican como muy tempranas, si maduran a principios de primavera; tempranas si su maduración es a finales de primavera; de estación media, cuando maduran a principios de verano; tardías si maduran a mediados de verano; y, para terminar, muy tardías cuando su maduración se alcanza a finales de verano.

Actualmente, el cultivo y consumo de arándanos en España está en aumento, es el tercer productor europeo por detrás de Alemania y Polonia. Según los datos encontrados de superficies y producciones anuales de cultivo de acuerdo con el Reglamento (CE) 543 / 2009 en el Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, en España en 2017 hay un total de 3260 ha de cultivo de arándanos con una producción de 35355 Tn. La provincia de Huelva es la mayor productora de arándano de España con una superficie cultivada de 2983 ha y una producción de 34077 Tn, además, es la mayor zona productora de Europa para cosecha temprana. Según las conclusiones que se han obtenido en las "*IV Jornadas Técnicas del Cultivo del Arándano en la Provincia de Huelva"* el 96 % de las exportaciones de la producción española van para Estados Unidos y el otro 4 % a países como Suiza, Malasia, Singapur, entre otros.

2. COMPUESTOS BIOACTIVOS EN UVAS Y ARÁNDANOS

Los compuestos bioactivos o fitoquímicos son sustancias químicas no nutritivas que pueden ser esenciales o no esenciales para el cuerpo humano. Se pueden encontrar en la naturaleza, de origen animal o vegetal; y sintéticos, total o parcialmente. Presentan funciones biológicas que pueden promover una

mejora en la salud. Algunos autores como Abdelkarim *et al.* definen los compuestos bioativos como "*un compuesto que tiene la capacidad y la habilidad para interactuar con uno o más componentes del tejido vivo presentando una amplia gama de efectos probables*" (Abdelkarim *et al.*, 2014), y otros como Fernandes *et al.* los definen como *"compuestos presentes en alimentos, animales, o plantas que tienen un efecto en el cuerpo que los consume*" (Fernandes *et al.*, 2019).

Como ya se ha mencionado los compuestos bioactivos pueden tener diferentes orígenes, pero en este trabajo nos vamos a centrar en los compuestos bioactivos presentes en los frutos rojos. Los frutos rojos, como la uva y el arándano, son ricos en compuestos fenólicos (Casagrande *et al.*, 2019; Guiné *et al.*, 2018) y en compuestos no fenólicos, como las vitaminas (Rodriguez-Mateos et al., 2019).

2.1. Compuestos fenólicos

Los compuestos fenólicos, metabolitos secundarios de las plantas, están presentes de forma natural en las frutas y vegetales, y son necesarios para su crecimiento y desarrollo. La especie, variedad, cultivo, región, condiciones climáticas, madurez, tiempo de cosecha, tiempo y condiciones de almacenamiento son factores de los que depende el contenido de compuestos fenólicos en frutas y verduras (Szajdek and Borowska, 2008).

Los frutos rojos en general tienen un alto contenido en compuestos fenólicos (Aleixandre-Tudo *et al.*, 2017; Borochov-Neori *et al.*, 2015; Skrovankova *et al.*, 2015). Su concentración es más alta en la piel de los frutos y en la zona más cercana a ella, que en el interior (Szajdek and Borowska, 2008). Un contenido elevado de estos compuestos puede presentar algunos inconvenientes, en particular los taninos (Szajdek and Borowska, 2008), que pueden inhibir la biodisponibilidad del hierro y la tiamina, pueden bloquear enzimas digestivas del tracto gastrointestinal y además, pueden formar complejos insolubles con las proteínas e inhibir su disponibilidad (Oh and Hoff, 1987; South and Miller, 1998; Tamir and Alumot, 1969; Wang and Kies,

1991). Estos compuestos están muy estudiados debido a la multitud de propiedades beneficiosas que presentan para la salud. Son antioxidantes capaces de eliminar especies reactivas de oxígeno (ROS) con efectos reductores del estrés oxidativo, lo que les da propiedades antiinflamatorias, cardioprotectoras, anticancerígenas, neuroprotectores, antienvejecimiento, protectores de los ojos, protectoras renales. Además tienen funciones contra la diabetes tipo 2 y dietéticas, reductoras de la obesidad (Aleixandre-Tudo *et al.*, 2017; Baby *et al.*, 2018; Das and Mandal, 2018; Fairlie-Jones *et al.*, 2017; Folmer *et al.*, 2014; Lee *et al.*, 2017; Leong *et al.*, 2018; Paredes-López *et al.*, 2010; Pavlidou *et al.*, 2018; Różańska and Regulska-Ilow, 2018; Singh *et al.*, 2018; Skrovankova *et al.*, 2015).

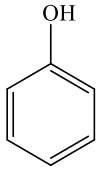


Figura 6. Estructura de un fenol.

Existen muchísimos compuestos fenólicos en la naturaleza. De todos ellos, se conoce que unos 8000 presentan una estructura común, un fenol, que es un anillo bencénico en el que se puede encontrar al menos un sustituyente hidroxilo (Figura 6). Pero pueden presentar una gran diversidad de estructuras, que se podrían clasificar en dos grandes grupos, los no flavonoideos, como son los ácidos fenólicos y estilbenos; y los flavonoideos, como los antocianos, flavonoles, flavanoles, flavonas, entre otros (Blancquaert *et al.*, 2019; Paredes-López *et al.*, 2010).

2.1.1. No flavonoideos

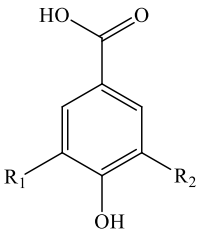
Los compuestos fenólicos no flavonoideos más importantes en los alimentos son los ácidos fenólicos, entre los que podemos distinguir los ácidos hidroxibenzoicos e hidroxicinámicos; y los estilbenos.

2.1.1.1. Ácidos fenólicos

Ácidos hidroxibenzoicos

Los ácidos hidroxibenzoicos están compuestos por 7 átomos de carbono con una estructura C6-C1, formados por un anillo bencénico y una cadena alifática. Incluyen los ácidos gálico, *p*-hidroxibenzoico, protocatéquico, vaníllico y siríngico (Barros *et al.*, 2009); recogidos en la Tabla 2.

Tabla 2. Estructura de los ácidos hidroxibenzoicos.

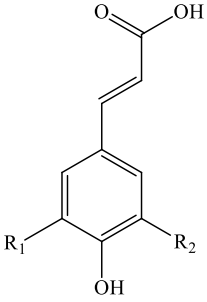


Ácido hidroxibenzoico	**R_1**	**R_2**
Ácido gálico	OH	OH
Ácido *p*-hidroxibenzoico	H	H
Ácido protocatéquico	OH	H
Ácido vaníllico	OCH_3	H
Ácido siríngico	OCH_3	OCH_3

Ácidos hidroxicinámicos

Los ácidos hidroxicinámicos están compuestos por 9 átomos de carbono con una estructura C6-C3, formados por un anillo bencénico y una cadena alifática de 3 carbones, caracterizada por contener un doble enlace. Esto permite a estos compuestos tener dos isómeros ópticos, *cis* y *trans*, siendo los más abundantes los isómeros *trans*. Incluyen los ácidos *p*-cumárico, cafeico, ferúlico y sinapínico (Barros *et al.*, 2009); recogidos en la Tabla 3. Estos ácidos se pueden encontrar en forma de ésteres del ácido tartárico, dando lugar a los ácidos caftárico (ácido cafeoiltartárico), cutárico (ácido *p*-cumariltartárico) y fertárico (ácido feruloiltartárico).

Tabla 3. Estructura de los ácidos hidroxicinámicos.



Ácido hidroxicinámico	**R_1**	**R_2**
Ácido *p*-cumárico	H	H
Ácido cafeico	OH	H
Ácido ferúlico	OCH_3	H
Ácido sinapínico	OCH_3	OCH_3

Los ácidos fenólicos son una de las familias de compuestos fenólicos más importantes en uvas blancas. En las uvas el ácido fenólico con un mayor contenido suele ser el derivado del ácido hidroxibenzoico llamado ácido gálico. Pero las uvas pueden contener otros ácidos como son ácido protocatéquico, ácido p-hydroxybenzonic, ácido clorogénico, ácido vaníllico, ácido cafeico, ácido siríngico, ácido *p*-cumárico, ácido ferúlico y ácido

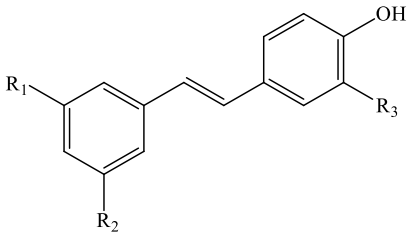
sinapínico (Huang *et al.*, 2009) y ésteres de los de los ácidos hidroxicinámicos (Spanos and Wrolstad, 1990). Autores como Gokturk Baydar cuantificaron un contenido de ácidos fenólicos totales entre 1.7 y 6.6 µg/g de las variedades de uva utilizadas (Gokturk Baydar, 2006).

Según el estudio de Sellappam *et al.* los arándanos "*rabbiteye"* analizados tenían un contenido de ácidos fenólicos totales entre 5.3 y 275.9 mg/100g fruta fresca, y para los arándanos "*southern highbush"* entre 10.9 y 18.5 mg/100g fruta fresca en los que se han podido identificar ácido gálico, *p*-hidroxibenzoico, cafeico, *p*-cumárico, ferúlico y elágico (Sellappan *et al.*, 2002). Otros estudios identifican como los ácidos fenólicos predominantes, el ácido cafeico y el ácido ferúlico (Huang *et al.*, 2012).

2.1.1.2. Estilbenos

Los estilbenos están compuestos por una estructura C6-C2-C6, formados por dos anillos bencénicos unidos por un puente etileno, que puede encontrase en dos formas isómeras, *cis* y *trans*, siendo esta última más estable. Los estilbenos presentan muchas funciones en las plantas, entre las que podemos encontrar que son compuestos antimicrobianos de bajo peso molecular, disuasivos y repelentes, protegiendo a las plantas de ataques fúngicos, bacterianos, de nematodos o de herbívoros (Aslam *et al.*, 2009; Jeandet *et al.*, 2010). Actualmente los estilbenos, en especial el *trans*-resveratrol (Tabla 4), han sido ampliamente estudiados por sus numerosas propiedades como cardioprotectoras, antitumorales, neuroprotectoras y agentes antioxidantes (Hung *et al.*, 2017; Jeandet *et al.*, 2010; Li *et al.*, 2017).

Tabla 4. Estructura de los estilbenos.



Estilbeno	**R_1**	**R_2**	**R_3**
Trans-resveratrol	OH	OH	H
pteroestilbeno	OCH_3	OCH_3	H
piceatannol	OH	OH	OH

En uvas y arándanos se han identificado *trans*-resveratrol y otros dos estilbenos, pteroestilbeno y piceatannol (Jeandet *et al.*, 2010; Rimando *et al.*, 2004). El estilbeno más estudiado en uva es el *trans*-resveratrol, que lo

podemos encontrar en fruta fresca y en los vinos elaborados, autores como Iacopini *et al.* han evaluado el contenido en este compuesto en diferentes tipos de uvas y se puede encontrar entre 0.7 y 25.5 mg/100 g de fruta seca (Iacopini *et al.*, 2008). Autores como Rimando *et al.* han demostrado que las especies *Vacinium corymbosum*, *Vacinium ashei* y *Vacinium angustifolium* contienen *trans*-resveratrol hasta 860 ng/g de fruta seca, las especies *Vacinium ashei* y *Vacinium stamineum* contienen pteroestilbeno en niveles entre 99-520 ng/g de fruta seca y las especies *Vacinium corymbosum* y *Vacinium stamineum* contienen piceatannol en niveles entre 138-422 ng/g de fruta seca (Rimando *et al.*, 2004).

2.1.2. Flavonoideos

Todos los flavonoideos presentan una estructura en común formada por 15 átomos de carbono en el esqueleto base de acuerdo con una estructura C6-C3-C6 (Figura 7), constituida por dos anillos aromáticos (anillo A y B) unidos por un heterociclo oxigenado, pirano (anillo C). Las diferentes familias de flavonoides (Figura 7) se pueden distinguir según las variaciones en su estructura, atendiendo al número de insaturaciones y los sustituyentes del anillo C.

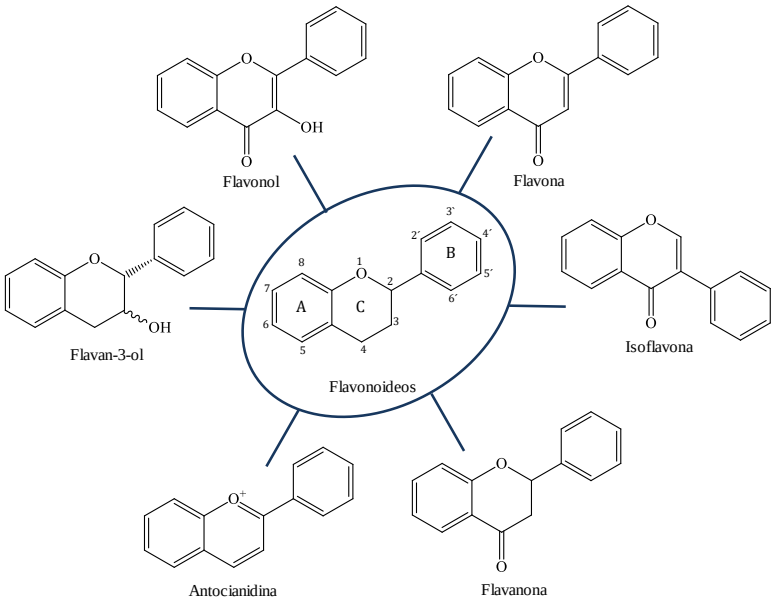


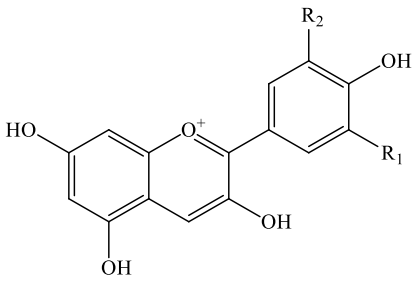
Figura 7. Estructura base de los flavonoideos y diferentes familias a partir de ella.

Los compuestos fenólicos flavonoideos son los compuestos fenólicos más abundantes en el reino vegetal, su concentración es más elevada en la epidermis de las hojas y de los frutos y tienen funciones importantes como metabolitos secundarios, entre las que podemos encontrar la protección frente a la radiación ultravioleta, la pigmentación de hojas y frutos, la resistencia contra enfermedades y la fijación del nitrógeno (Cartea *et al.*, 2011; Prasad *et al.*, 2009).

2.1.2.1. Antocianos

Las antocianidinas o agliconas hacen referencia a la estructura básica de los antocianos o antocianinas, con un esqueleto C6-C3-C6 común con todos los compuestos flavonoideos. Los antocianos son colorantes naturales responsables de los colores naranja, rosa, rojo, violeta y azul de muchas plantas vasculares (Liu *et al.*, 2019). En la naturaleza se pueden encontrar hasta 23 antocinidinas (Castañeda-Ovando *et al.*, 2009), que difieren en el grado de hidroxilación y/o metoxilación de la estructura básica. Las más comunes en frutas y verduras las podemos ver en la Tabla 5 y son cianidina, delfinidina, pelargonidina, peonidina, petunidina y malvidina (Clifford, 2000).

Tabla 5. Agliconas de las antocianinas.



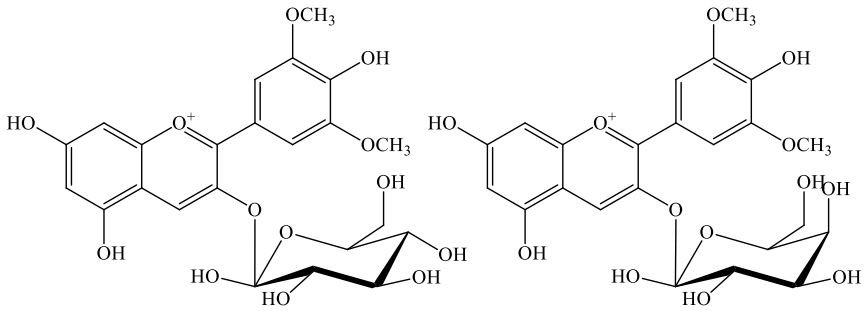
Antocianidina	**R_1**	**R_2**
Cianidina	OH	H
Delfinidina	OH	OH
Pelargonidina	OH	OCH_3
Peonidina	OCH_3	H
Petunidina	OCH_3	OH
Malvidina	OCH_3	OCH_3

Las antocianidinas son derivados del ion flavilio y debido a la deficiencia electrónica del catión son altamente reactivas, por lo que rara vez se encuentran en la naturaleza como agliconas (Clifford, 2000). Los antocianos son heterósidos de las antocianidinas, resultado de la unión de una aglicona con una molécula de azúcar por un enlace glucosídico. Los azúcares más comunes en la formación de antocianos son glucosa, galactosa, ramnosa, arabinosa y xilosa, siendo la glucosa la mayoritaria (Andersen and Jordheim,

2010). En la naturaleza no solo se encuentran derivados monoglucosilados de las agliconas, sino que también podemos encontrar derivados diglucosilados y triglucosilados. Además, también existen derivados acetilados de los monoglucósidos con ácidos orgánicos como el ácido acético, *p*-cumárico y cafeico.

El antociano mayoritario en las uvas es el malvidin-3-*O*-glucósido (Figura 8) (Marquez et al., 2012b, 2013; MohdMaidin et al., 2019). Según los autores Andersen *et al.* los derivados de antocianidinas que se pueden encontrar en las uvas son: malvidina, cianidina, peonidina, petunidina y delfinidina 3-*O*-glucósico, 3,5-diglucósido, 3-*O*-(6-p-cumaril-glucósido), 3-*O*-(6-acetil-glucósido) y 3-(6'-p-coumaril-glucósido) 5-glucósido (Andersen and Jordheim, 2010). En las uvas los antocianos están principalmente presentes como derivados 3-*O*-glucosilados, debido a una mutación en el gen de la encima 5-*O*-glucosiltransferasa que impide su funcionalidad (Ferreira *et al.*, 2018). De las 6 agliconas más comunes, la pelargonidina no está presente en las uvas (Mattivi *et al.*, 2006). Aunque autores como Marquez *et al.* identifican pelargonidin 3-*O*-glucósido en mostos de uva de las variedades Merlot y Syrah (Marquez *et al.*, 2012a), y autores como Rodrigues *et al.* lo detectaron en una nueva variedad de uva híbrida cultivada en Brasil (Rodrigues da Silva *et al.*, 2019).

Los antocianos pueden representar hasta un 70 % de los compuestos fenólicos de los arándanos (Olas, 2018). En los arándanos se han determinado derivados de las agliconas cianidina, delfinidina, pelargonidina, petunidina, peonidina y malvidina (Chaves *et al.*, 2018; Routray and Orsat, 2011), algunos autores proponen que el antociano mayoritario de los arándanos es el malvidin 3-O-galactósido (Figura 8) (Skrede *et al.*, 2000; Varo *et al.*, 2018). Sin embargo, otros afirman que es el delfinidin 3-O-galactósido (Mi *et al.*, 2004). En arándanos podemos encontrar derivados glicosilados de glucosa, galactosa y arabinosa, y derivados acetilados en el carbono 6 del azúcar, del ácido orgánico p-cumárico (Stevenson and Scalzo, 2012). En un estudio que se realizó a 80 genotipos diferentes de arándano, entre las temporadas de 2009 y 2012 en Nueva Zelanda, presentaban un contenido en antocianos en el rango de 57-503 mg/100g.



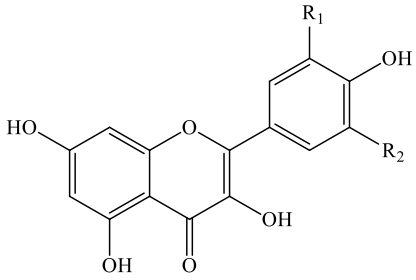
Malvidin 3-*O*-glucósido Malvidin 3-*O*-galactósido

Figura 8. Estructuras del malvidin 3-*O*-glucósido y malvidin 3-*O*-galactósido.

2.1.2.2. Flavonoles

Los flavonoles presentan el esqueleto C6-C3-C6 y los caracteriza el doble enlace entre las posiciones de los carbonos 2 y 3. Pueden recibir el nombre de 3,5,7-trihidroxiflavonas, puesto que el 90% de los flavonoles poseen un grupo hidroxilo en estas posiciones (Castillo-Muñoz *et al.*, 2007). Los flavonoles son la familia de compuestos flavonoideos más común en las plantas (Marzocchella *et al.*, 2011) y las 6 agliconas disponibles, recogidas en la Tabla 6, son isorhamnetina, kaempferol, laricitrina, miricetina, quercetina y siringetina (Gómez-Alonso *et al.*, 2018). Los flavonoles se suelen encontrar en las plantas como *O*-glucósidos, más frecuentemente de los azúcares glucosa y ramnosa; aunque también se pueden ver heterósidos de galactosa, arabinosa y xilosa, e incluso derivados acetilados del ácido glucurónido (Marzocchella *et al.*, 2011)

Tabla 6. Agliconas de los flavonoles.



Flavonol	**R_1**	**R_2**
Isorhamnetina	OCH_3	H
Kaempferol	OH	H
Laricitrina	OCH_3	H
Miricetina	OH	OH
Quercetina	OH	H
Siringerina	OCH_3	OCH_3

Los flavonoles están en mayor concentración en la piel de los frutos, son pigmentos naturales de color amarillo debido al máximo de absorción que presentan aproximadamente a 350 nm y 370 nm, para los glicósidos y agliconas respectivamente.

Los derivados de la aglicona quercetina son los predominantes en las plantas (Marzocchella *et al.*, 2011). En uvas blancas se pueden identificar derivados 3-*O*-glucósidos y 3-*O*-galactósidos de kaempferol, quercetina e isorhamnetina, así como los 3-*O*-glucurónidos de kaempferol y quercetina (Castillo-Muñoz *et al.*, 2010). En uvas tintas se pueden identificar derivados 3-*O*-glucósidos de las 6 agliconas, 3-*O*-glucurónidos de miricetina, quercetin y kaempferol, 3-*O*-galactósidos de kaempferol y laricitrina y 3-(6'-acetil)glucósidos de la quercetina y la siringetina (Castillo-Muñoz *et al.*, 2007). En un estudio realizado por Mattivi *et al.*, en el que se analizó el contenido de los flavonoles en la piel de 64 variedades de uva tinta, se identificaron derivados de las 6 agliconas, quecertina (43.99%), miricetina (36.81%), kaempferol (6.43%), laricitrina (5.65%), isorhamnetina (1.74%) y siringetina (3.22%), con un contenido total comprendido entre 3.81 y 78.26 mg/kg; y en la piel de 27 variedades de uva blanca se identificaron derivados de tres agliconas, quercetina (81.35%), kaempferol (16.91%) y isorhamnetina (1.74%), con un contenido total en el rango 1.36-30.91 mg/kg (Mattivi *et al.*, 2006).

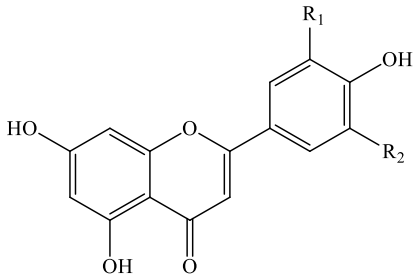
En arándanos se han podido identificar derivados 3-*O*-glucósidos de quercetina, kaempferol, siringetina y miricetina, 3-*O*-galactósido de quercetina, 3-*O*-rutinósido de quercetina, 3-*O*-arabinósido de quercetina, 3-*O*-ramnósido de miricetina y quercetina, 3-*O*-glucurónido de quercetina (Girones-Vilaplana *et al.*, 2016; Grace *et al.*, 2019; Mlcek *et al.*, 2016; Skrovankova *et al.*, 2015). Estos compuestos se pueden encontrar en arándanos en un rango de concentración entre 24 – 28.5 mg/kg fruta fresca (Bilyk and Sapers, 1986; Häkkinen *et al.*, 1999).

2.1.2.3. Flavonas

Las flavonas comparten el esqueleto C6-C3-C6 con el resto de los flavonoides, pero se caracterizan por el doble enlace que presentan en los carbonos 2 y 3, y por el grupo cetona que posee en la posición 4. Las agliconas de las flavonas más abundantes en el reino vegetal son apigenina, luteolina y tricina (Tabla 7).

En las uvas se ha identificado una aglicona de las flavonas, luteolina, con un contenido entre 0.1 y 2.6 mg/100g de fruta fresca. Sin embargo, en vinos blancos y tintos se han determinado dos agliconas de las flavonas, apigenina y luteolina, con una concentración menor a 1 mg/100mL de vino. En los arándanos sólo se ha identificado la aglicona luteolina en un rango de 1.2 – 1.8 mg/100 g de fruta fresca (Hostetler *et al.*, 2017).

Tabla 7. Agliconas de las flavonas.



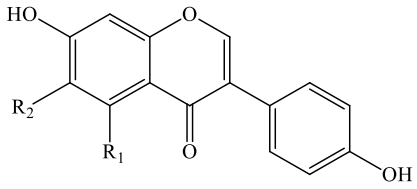
Flavona	**R_1**	**R_2**
Apigenina	OH	H
Luteolina	H	H
Tricina	OH	OH

2.1.2.4. Isoflavonas

Las isoflavonas son isómeros de las flavonas, puesto que el anillo aromático B se encuentra en la posición 3. Las agliconas de las isoflavonas más comunes (Tabla 8) son daidceína, genisteína y en menor contenido gliciteína (Marzocchella *et al.*, 2011). Las isoflavonas se encuentran principalmente en las leguminosas, los niveles más altos se pueden encontrar en la soja y sus derivados (Swart *et al.*, 2019).

Las isoflavonas están presentes en uvas y vinos pero a una concetración baja, autores como De Sanctis *et al.* han podido identificar daidceína y genisteína, en la deshidratación de uva blanca para vinificación (De Sanctis *et al.*, 2012).

Tabla 8. Agliconas de las isoflavonas.

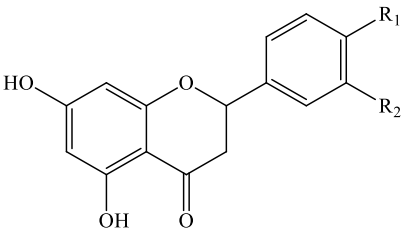


Isoflavona	**R_1**	**R_2**
Daidceína	H	H
Genisteína	OH	H
Gliciteína	H	OCH_3

2.1.2.5. Flavanonas

Las flavanonas presentan el esqueleto C6-C3-C6, pero su estructura deriva de la reducción de las flavonas, presentando un centro quiral en el C2. En la naturaleza, normalmente, se encuentran con una configuración α del C2 entre el anillo B y C. Las flavanonas se suelen encontrar en frutas cítricas, donde las agliconas más comunes son, naringenina, hesperidina, y eriodictiol (Tabla 9) (Martínez-Navarrete *et al.*, 2008; Marzocchella *et al.*, 2011).

Tabla 9. Agliconas de los flavanonas.

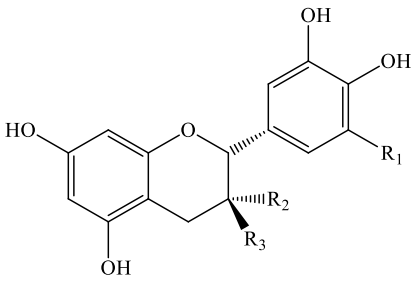


Flavanona	**R_1**	**R_2**
Eriodictiol	OH	OH
Hesperidina	OCH_3	OH
Naringenina	OH	H

2.1.2.6. Flavan-3-oles

Los derivados del flavan-3-ol, flavan-3-oles o flavanoles presentan el esqueleto básico C6-C3-C6, pero difieren del resto de flavonoides al tener un anillo C casi saturado con un grupo hidroxilo adicional en la posición 3, que como centro quiral da lugar a los isómeros ópticos, (+) y (-). Los monómeros más comunes son 2 pares de estereoisómeros: (+)-catequina, (-)-epicatequina, (+)-galocatequina y (-)-epigalocatequina (tabla 10). Aunque también pueden aparecer en las plantas esterificados con ácido gálico: (+)-catequín 3-*O*-galato, (-)-epicatequín 3-*O*-galato, (+)-galocatequín 3-*O*-galato y (-)-epigalocatequín 3-*O*-galato (Suroviec *et al.*, 2019).

Tabla 10. Agliconas de los flavan-3-oles



Flavan-3-ole	**R_1**	**R_2**	**R_3**
(+)-Catequina	H	H	OH
(-)- Epicatequina	H	OH	H
(+)-Galocatequina	OH	H	OH
(-)-Epigalocatequina	OH	OH	H

Los flavan-3-oles son la familia de compuestos flavonoideos más abundante en la uva. Los monómeros más abundantes en las semillas de uva son la (+)-catequin, (-)-epicatequina y (-)-epicatequín 3-*O*-galato. Mientras que en la piel de la uva también se puede encontrar (-)-epigalocatequina y (+)-galocatequina (Blancquaert *et al.*, 2019). Autores como Huang *et al.* han detectado en muestras de vino de uva 5 monómeros: (+)-catequin, (-)-epicatequina, (-)-epigalocatequina, (-)-epicatequín 3-*O*-galato y epigalocatequín 3-*O*-galato (Huang *et al.*, 2009). En uvas blancas se ha cuantificado un contenido total de flavan-3-oles de de 1 mg/100 g fruta fresca y en uvas tintas de 0.8 mg/g fruta fresca (Gu *et al.*, 2004).

En los arándanos se han podido identificar 2 monómeros de los flavan-3-oles, entre los que están la (+)-catequina y (-)-epicatequina, siendo la catequina el mayoritario (Grace *et al.*, 2019; Song and W. Olmstead, 2016). Los arándanos pueden presentar un contenido total comprendido entre 174.8 y 331.9 mg/100 g de fruta fresca (Marzocchella *et al.*, 2011).

2.1.2.7. Taninos

Los taninos son compuestos fenólicos de alto peso molecular, en un rango entre 500 y 3000 Da (Asho and Upadhyaya, 2012), que dan lugar a multitud de estructuras químicas. Estos compuestos tienen números efectos beneficiosos para la salud debido a su capacidad antioxidante (Gourlay and Constabel, 2019). Otra propiedades que se atribuyen a los taninos es la astringencia, por su capacidad de establecer combinaciones estables con las proteínas y hacer que precipiten (Bate-Smith, 1973; Peña-Neira, 2019; Ployon *et al.*, 2018) y la posibilidad de formar complejos con iones metálicos (Schofield et al., 2001).

Estos polímeros se pueden dividir en tres grandes grupos dependiendo de la naturaleza de los monómeros: taninos condensados, taninos hidrolizables y taninos complejos.

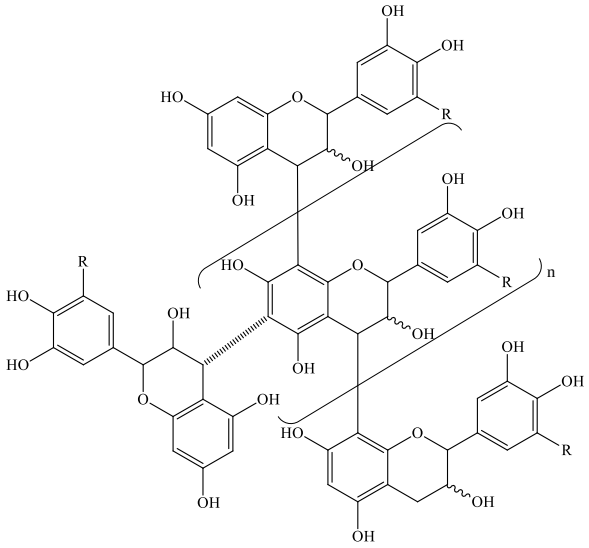
Taninos condensados

Los taninos condensados, de origen flavonoide, son el resultado de la polimerización de unidades de flavan-3-ol. Se pueden encontrar denominadas también como proantocianidinas, debido a la obtención de antocianinas tras su hidrólisis ácida. Según la antocianina que se libere se pueden denominar procianidinas o prodelfinidinas (Tabla 11), si el antociano que resulta de la

hidrólisis es cianidina o delfinidina, respectivamente. Las unidades monoméricas de flavan-3-oles relacionadas con las procianidinas son catequina o epicatequina, mientras que con las prodelfinidinas son galocatequina o epigalocatequina (Grabber *et al.*, 2013).

Los taninos condensados se encuentran en la piel y las semillas de uvas y arándanos, pero no en la pulpa (Schofield et al., 2001). Numerosos autores han identificado estos compuestos en uvas (de Oliveira et al., 2019; Jordão and Ricardo-da-Silva, 2019; Schofield et al., 2001) y arándanos (Gu *et al.*, 2002; X. Li *et al.*, 2019; Naczk *et al.*, 2006).

Tabla 11. Estructura de las proantocianidinas.



Proantocianidina	R
Procianidina	H
Prodelfinidina	OH

Taninos hidrolizables

Los taninos hidrolizables, de origen no flavonoide, son el resultado de la polimerización de ésteres de ácidos fenólicos con azúcares. Tras su hidrólisis ácida aparecen sus unidades monoméricas que pueden ser ácido gálico o elágico, donde los taninos recibirían el nombre de galotaninos o elagitaninos respectivamente (Niemetz and Gross, 2005).

Los taninos son un importante componente de las frutas tipo baya, pero los taninos hidrolizables son los menos frecuentes y se han podido identificar en fresas, frambuesas y moras (Szajdek and Borowska, 2008). Sin embargo, en vinos que han pasado por madera si se pueden identificar. Esto es debido a

que la madera principal en el envejecimiento en barrica es de roble, y el grupo de taninos más abundante en este tipo de madera son los hidrolizables (Puech *et al.*, 1999). Estos compuestos son solubles en agua y se disuelven fácilmente en disoluciones hidroalcohólicas, como el vino, por lo que se pueden extraer durante el envejecimiento (Mcrae and Kennedy, 2011).

Taninos complejos

Los taninos complejos o mixtos son el resultado de la combinación de los taninos condensados e hidrosolubles (Ployon *et al.*, 2018), por uniones covalentes entre los ácidos fenólicos y los flavan-3-oles.

2.2. Compuestos no fenólicos

Otros compuestos bioactivos no fenólicos son las vitaminas. Las vitaminas son sustancias químicas no sintetizables por el organismo (excepto vitamina D y K), presentes en pequeñas cantidades en los alimentos y que son indispensables para el correcto funcionamiento del cuerpo. Las vitaminas se pueden clasificar en dos grupos atendiendo a su solubilidad: liposolubles que son aquellas que se disuelven en grasas y aceites, como la vitamina A, D, E, K y F; e hidrosolubles si se disuelven en agua, como las vitaminas del complejo B y la vitamina C.

2.2.1. Vitamina C

La vitamina C es del tipo hidrosoluble, esta tiene una alta capacidad antioxidante y puede actuar como cofactor de numerosas enzimas en reacciones redox. Además, se ha demostrado que posee un papel importante en los mecanismos que regulan la diferenciación celular, cuya desregulación podría desembocar en diferentes tipos de cáncer (Fenech *et al.*, 2019).

Vitamina C es el descriptor que se utiliza para todos los derivados del ascorbato que tienen actividad biológica, entre ellos tenemos el ácido L- ascórbico, el ácido L-dehidroascórbico y los ésteres del ácido ascórbico. El ácido L-ascórbico es la forma reducida de la vitamina C, por lo tanto, si nos encontramos en condiciones oxidativas, esta se convierte fácilmente en ácido L-dehidroascórbico (Amirah Hair Mustapa and Rohaiza Ahmad, 2019) por medio de la formación de radicales (Figura 9). Lo que según autores como Nyyssönen *et al.* podría explicar la capacidad antioxidante de estos compuestos (Nyyssönen *et al.*, 2000).

La vitamina C la podemos encontrar en frutas y verduras, las más conocidas por ello son los cítricos. Las uvas no se caracterizan por su alto contenido en vitamina C, pero también se ha detectado a una concentración entre 3 – 13 mg/100 g de frusta fresca (Hägg *et al.*, 1995) o en zumos de uva blanca entre 4.80 y 19.23 mg/dL (Kovaleski *et al.*, 2019). Sin embargo, los arándanos si son ricos en vitamina C, autores como Kim *et al.* la han detectado con una concentración de 969.9 mg/100 mL (Kim *et al.*, 2018) y autores como Sun *et al.* que estudiaron la composición en vitamina C en 14 cultivos de arándanos establecieron un rango de concentración entre 150 y 239 mg/100g de fruta fresca (Sun *et al.*, 2017).

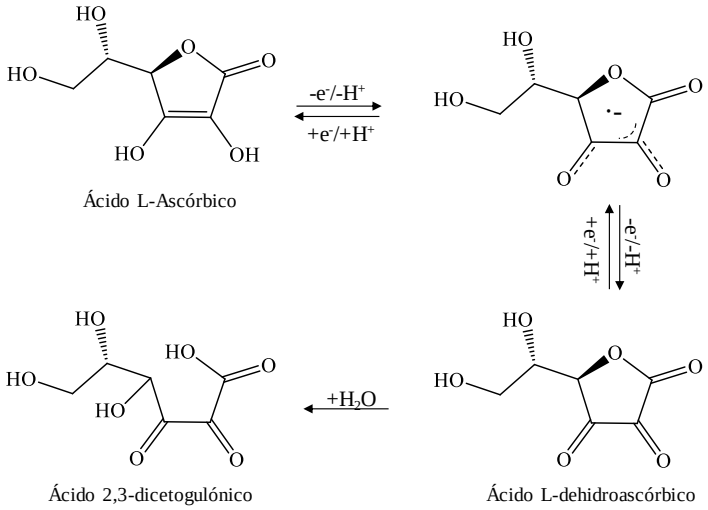


Figura 9. Estructura de la forma oxidada y reducida de la vitamina C.

3. SECADO

3.1. Definición de secado

El secado se puede definir como el proceso de eliminación térmica de humedad para producir un producto seco. Cuando un producto es sometido a un secado térmico se llevan a cabos dos procesos de transferencia simultáneos de calor y masa. El primero, la transferencia de energía térmica del entorno al producto para evaporar la humedad superficial; y el segundo, la transferencia de la humedad interna a la superficie del producto y su posterior evaporación debido al primer proceso (Mujumdar, 2007).

La transferencia de energía, en forma de calor, se puede llevar a cabo por diferentes mecanismos, como son conducción, convección y radiación, o por la combinación de ellos (K. Li *et al.*, 2019). En la conducción (Figura 10 A) la energía térmica se transfiere por contacto directo entre superficies calentadas y el material a secar (Noomhorm *et al.*, 1994), la humedad liberada se elimina con el uso de vacío o de corriente de gases que la arrastren. En la convección (Figura 10 B), la transferencia de energía se hace mediante corrientes de aire caliente que pasan a través del material a secar, que además de transmitir el calor, también actúa como transportador de la humedad liberada (Vallespir *et al.*, 2019). Por último, la transferencia de energía por radiación (Figura 10 C), se debe a la transmisión de calor por ondas electromagnéticas, incluyendo varias fuentes de radiación electromagnética como pueden ser microondas, radio frecuencia, infrarrojo y ultrasonidos (K. Li *et al.*, 2019).

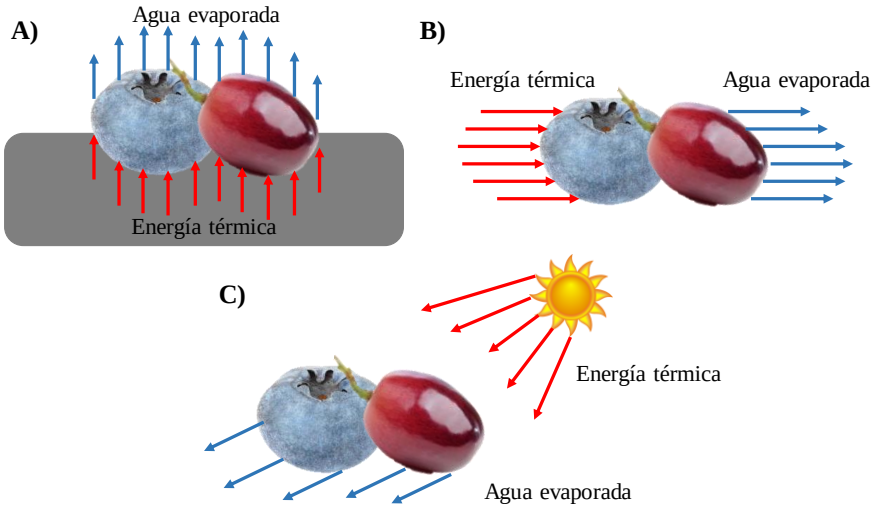


Figura 10. Mecanismos de transferencia de energía. (A) conducción, (B) convección y (C) radiación.

3.2. Secado de alimentos

Los procesos de secado son métodos de conservación de alimentos, con los que se consigue alargar la vida útil de los productos puesto que se disminuye la actividad de agua lo suficiente como para evitar la proliferación de

microorganismos, las reacciones enzimáticas y otras reacciones de deterioro (Doymaz, 2017).

Según los autores Ratti and Mujumdar las características necesarias en los alimentos a secar son un alto contenido de humedad inicial, alta sensibilidad a la temperatura, alta susceptibilidad al ataque microbiano y poseer una piel que permita la transferencia de agua o humedad (Ratti and Mujumdar, 1997). Haciendo referencia a estas características, los frutos rojos las cumplen todas y serían perfectos para su deshidratación.

3.2.1 Secado al sol

El secado al sol es el método de conservación de alimentos más antiguo, era usado en todo el mundo para secar plantas, frutas, semillas, carne, pescado y otros productos agrícolas (Baker, 1997). Aún se sigue utilizando en la deshidratación de las frutas para su conservación o con el objetivo de obtener nuevos productos. El secado de frutas al sol está aún en uso sin prácticamente cambios, en muchos países tropicales y subtropicales (Esper and Mühlbauer, 2002).

Desde el punto de vista del sector enológico, es muy importante puesto que existen muchos tipos de vinos que se elaboran a partir de uvas deshidratadas. Históricamente, la producción de pasas por secado de las uvas al sol se remonta a 1490 a.C. en Grecia (Jairaj *et al.*, 2009) y se sigue utilizando en países productores de uva. En el sur de España, en la denominación de origen Montilla-Moriles de la provincia de Córdoba, es una práctica aún vigente. Se lleva a cabo la pasificación de la uva Pedro Ximenez para la elaboración de su vino dulce natural, que recibe el nombre de la variedad de uva (Ruiz *et al.*, 2009).

En el secado tradicional al sol o “asoleo”, los racimos de uvas se disponían en capachos de esparto en el suelo, pero actualmente se distribuyen por grandes mayas de plástico como se puede observar en la Figura 11. Todos los días es necesario el volteo de los racimos, para que la radiación incida en todas las bayas y el contenido de los compuestos sea uniforme. Este proceso se puede extender entre 5 y 10 días, dependiendo de las condiciones climáticas (Serratosa *et al.*, 2008), hasta que se pierda aproximadamente un 50 % de la humedad. En la Figura 11 se puede ver el estado inicial y final de las uvas durante el proceso de secado al sol.

El problema de este tipo de secado es que presenta numerosos inconvenientes, como puede ser el tiempo prolongado del proceso, la dependencia de los factores climáticos (lluvia, intensa radiación solar, cambios de temperatura entre el día y la noche, …), el ataque de insectos, la pérdida de material por pájaros u otros animales, las contaminaciones microbianas y la descomposición de los granos. Estos inconvenientes derivarían en un deterioro y una peor calidad de los productos finales (Jairaj *et al.*, 2009; Ratti and Mujumdar, 1997; Shende and Datta, 2019; Vijayavenkataraman *et al.*, 2012).

Figura 11. Progresión de la uva Pedro Ximenez en el secado al sol.

3.2.2. Secado por convección

Como alternativa al secado al sol, se puede utilizar el secado por convección forzada con aire caliente en cámaras o cabinas de secado, que permiten la aplicación de aire a una amplia superficie facilitando la evaporación del agua desde el material (Ruiz *et al.*, 2009). El secado por convección con aire caliente se utiliza normalmente en el secado de frutas y verduras (Vallespir *et al.*, 2019).

Este tipo de secado permite solventar los inconvenientes que tiene el secado al sol, con el que se reduce el tiempo de secado, permite establecer una temperatura y humedad relativa constantes, evita las contaminaciones y los ataques por animales (Karabulut *et al.*, 2006; Marquez *et al.*, 2014; Serratosa

et al., 2008). El inconveniente que presenta es que a diferencia del "asoleo" que es un secado muy barato, este requiere de un alto rendimiento para que sea rentable (Serratosa *et al.*, 2008), ya que es preciso el uso de energía eléctrica para su funcionamiento.

En la Figura 12 se puede ver el diagrama general del funcionamiento de una cámara de secado que esquematizaron los autores Serratosa *et al.* (Serratosa *et al.*, 2008). Donde una corriente de aire caliente con una temperatura constante incide sobre la muestra a desecar, el aire húmedo se extrae, se deshumidifica y vuelve a entrar caliente.

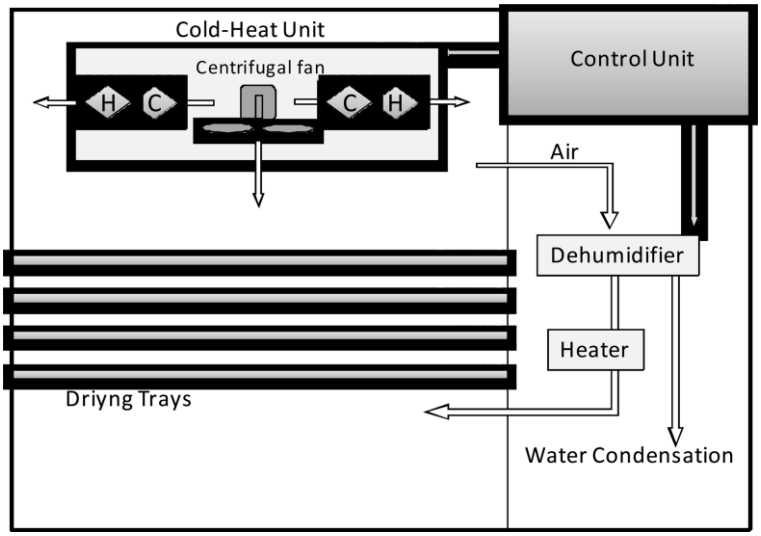


Figura 12. Diagrama de una cámara de secado (Serratosa *et al.*, 2008).

Para aumentar el rendimiento del secado convectivo en cámara se pueden emplear metodologías de tratamiento previas en el alimento. El tratamiento por congelación de las muestras antes del secado permite reducir el tiempo de secado un 13% en grosella, un 46% en banana, un 32% en remolacha y un 28% en manzana (Vallespir *et al.*, 2019); el tratamiento por inmersión de las muestras en una disolución alcalina, reduce el tiempo de secado un 24% en uva (Serratosa *et al.*, 2008); el tratamiento por deshidratación osmótica, puede reducir el tiempo de secado en un 48% en la deshidratación de yacon (de Oliveira *et al.*, 2016); el tratamiento de inmersión en disoluciones de sulfitos permiten reducir el tiempo de secado de albaricoques entre un 4 y 39 % (Karabulut *et al.*, 2006); entre muchos otros.

3.3. Compuestos bioactivos en el secado

Durante el proceso de secado se pueden dar reacciones de degradación de compuestos sensibles a la temperatura, se contrae el alimento por la pérdida de agua y se pueden dar reacciones de pardeamiento enzimático y no enzimático (Y. Sun *et al.*, 2019). Esto conlleva un deterioro de la calidad y de las propiedades del alimento a secar.

Los compuestos bioactivos no fenólicos, como la vitamina C, y los compuestos bioactivos fenólicos, como los ácidos fenólicos y los flavonoides, son sensibles a la temperatura, por lo que altas temperaturas de secado promueven su degradación (Amirah Hair Mustapa and Rohaiza Ahmad, 2019; Lang *et al.*, 2019). Los antocianos, responsables del color característico de los frutos rojos, como la uva y el arándano (objeto de estudio de este trabajo), es la familia fenólica más importante en los vinos tintos debido a que son los responsables de su color, se ha demostrado que son inestables a la temperatura (Sasongko *et al.*, 2019). Simpson propuso que la degradación térmica de los antocianos se podía dar por dos vías. La primera sería la hidrólisis del enlace 3-glucósido para formar la aglicona más lábil; y la segunda la hidrólisis y apertura del anillo pirilio para formar una calcona sustituida, que se degrada a un compuesto insoluble de color marrón y naturaleza polifenólica (Simpson, 1985).

El pardeamiendo enzimático de los frutos durante su procesado, es responsabilidad directa de los compuestos fenólicos y de la enzima polifenol oxidasa (PPO) (Lee *et al.*, 1990), que provoca cambios en el color, el sabor y la composición nutricional del alimento. Este tipo de reacciones son oxidaciones de los compuestos fenólicos en presencia de oxígeno (Whitaker and Lee, 1995), aunque los compuestos fenólicos se encuentran en las vacuolas de las células, cuando estas se rompen durante el proceso de secado, entran en contacto con el oxígeno. El pardeamiento no enzimático es otro tipo de oxidaciones, por ejemplo la reacción de Maillard, en la que se condensa el grupo carbonilo de los azúcares reductores con un grupo amino de los aminoácidos, proteínas o péptidos (Iyengar and McEvily, 1992). A diferencia del pardeamiento enzimático que necesita estar en presencia de oxígeno, el pardeamiento no enzimático puede trabajar en condiciones de anaerobiosis (Es-Safi *et al.*, 2000).

El proceso de secado puede aumentar el contenido de compuestos bioactivos en los alimentos debido al efecto de evaporación del agua, la hidrólisis de los flavonoides glucosilados y a la liberación de los fenoles de las paredes celulares (Udomkun *et al.*, 2015). Pero como se ha comentado anteriormente el uso de temperaturas elevadas puede hacer que estos compuestos se degraden. Autores como Stojanovic *et al.* han demostrado la pérdida de un 60% del contenido de antocianos de arándanos secos frente al contenido de arándanos frescos (Stojanovic and Silva, 2007); Yuan *et al.* estudiaron el secado a 65 ºC de 8 variedades distintas de arándanos y comprobaron que también se perdía un 60 % del contenido de antocianos (Yuan *et al.*, 2011). A pesar de que por encima de los 40 ºC disminuye la actividad de la PPO, un aumento de la temperatura de secado se traduce como una disminución más rápida de los compuestos fenólicos (Djendoubi Mrad *et al.*, 2012).

El secado convectivo por aire caliente en cámara es una buena alternativa al secado al sol, puesto que presenta un control de temperatura constante que permite controlar la pérdida de compuestos bioactivos.

4. FERMENTACIÓN ALCOHÓLICA

Las bebidas alcohólicas son todas aquellas que en su composición contienen etanol. Entre ellas se pueden distinguir las bebidas destiladas, como el ron, el whisky, el vodka, …; y las bebidas fermentadas, como el vino y la cerveza.

Las bebidas fermentadas como su nombre indica, son aquellas que realizan la fermentación alcohólica, que es la trasformación de azúcares, mayormente glucosa y fructosa, para la producción de etanol y dióxido de carbono (Zamora, 2009). Entre 1743 y 1794 fue cuando Lavoisier estableció que durante la fermentación alcohólica los azúcares se transformaban en alcohol, dióxido de carbono, ácidos orgánicos y ácido acético. Posteriormente, Gay-Lussac comprobó que el oxígeno gaseoso era un requisito necesario en este proceso y estableció la siguiente ecuación para el proceso (Frey, 1930):

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_6O + 2\ CO_2 + 57\ kcal/mol$$

Estos resultados fueron probados posteriormente por Pasteur con el estudio de las levaduras y las bacterias anaerobias (Sebald and Hauser, 1995), hasta la actualidad donde se cree que la formula correcta de la fermentación es:

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_6O + 2\ CO_2 + 33\ kcal/mol$$

La fermentación es una reacción exotérmica más compleja de lo explicado, al mismo tiempo se pueden dar muchos otros procesos químicos, bioquímicos y fisicoquímicos. Además del etanol, se pueden producir simultáneamente alcoholes superiores, ésteres, glicerol, ácido succínico, …(Zamora, 2009).

4.1. Crecimiento de levaduras

Las levaduras son las responsables de la transformación de los azúcares en gas carbónico y etanol, en el proceso denominado fermentación alcohólica. Las levaduras son organismos unicelulares que pertenecen al reino de los hongos, presentes en la piel de las bayas. Existen numerosos géneros y especies de levaduras en las frutas, pero las más comunes en la fermentación alcohólica son las especies del género *Saccharomyces.* Siendo la especie S*accharomyces cerevisiae* la más utilizada, por su resistencia a altas concentraciones de alcohol y a los sulfitos. Aunque pueden ser empleadas en la fermentación otras muchas especies como *Saccharomyces bayanus, Saccharomyces uvarum,* entre otras, o hibrídos de varias especies (González *et al.*, 2006).

El crecimiento de la levadura se constituye en cuatro fases:

Fase de latencia: Es un período de transición en el que los microorganismos se adaptan a unas nuevas condiciones. Esta es una fase previa al aumento en número de las células.

Fase exponencial: Esta fase se caracteriza por el crecimiento exponencial de los microorganismos, donde cada determinado tiempo se duplica la población. Durante esta fase las levaduras comienzan a fermentar los azúcares, principalmente glucosa y fructosa, a través de la glucólisis y comienza la liberación de etanol (Rezaei *et al.*, 2014).

Fase estacionaria: Esta fase se caracteriza por las limitaciones del crecimiento de las levaduras, que puede ser debido a una falta de nutrientes (azúcar), por la acumulación de productos tóxicos para los microorganismos (alcohol) o por una combinación de ambas.

Fase de muerte: Cuando acaba la fase anterior comienza a experimentar una merma en el número de células viables, por lo que se dice que las levaduras entran en fase de muerte.

4.2. Proceso de fermentación

La primera fase de la fermentación alcohólica es la degradación de los azúcares a través de la ruta metabólica llamada glucólisis, que consiste en una serie de reacciones catalizadas enzimáticamente. Esta ruta metabólica consigue degradar una molécula de glucosa o fructosa dando lugar a dos moléculas de piruvato. Pero los frutos no solo presentan los monosacáridos, glucosa y fructosa, sino también sacarosa que es un disacárido formado por ambos. Por este motivo, tiene que ser hidrolizado en sus monosacáridos (Figura 13), siendo la invertasa la enzima responsable de catalizar la reacción de ruptura de la sacarosa.

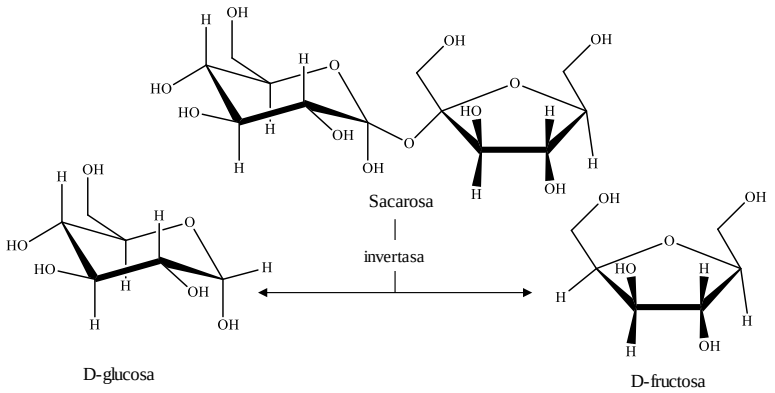


Figura 13. Hidrólisis de la sacarosa.

Según Nelson and Cox el proceso de degradación de la glucosa hasta piruvato comprende 10 pasos (Nelson and Cox, 2018):

Fosforilación de la glucosa y la fructosa

Es el primer paso de la glucólisis, en el que la glucosa y la fructosa son activadas para las siguientes reacciones con una fosforilación en el carbono C6. El dador del grupo fosforilo es el ATP (Figura 14), es una reacción irreversible y catalizada por la enzima hexoquinasa.

Conversión de la glucosa 6-fosfato en fructosa 6-fosfato

Este paso consiste en la isomerización reversible de una aldosa, glucosa 6-fosfato, en una cetosa, fructosa 6-fosfato (Figura 14). Esta reacción esta catalizada por la enzima fosfoglucosa isomerasa. La isomerización tiene un carácter crítico en la ruta metabólica, puesto que la posición de los grupos carbonilo e hidroxilo de los carbonos C1 y C2 son claves para los siguientes pasos de la glucólisis

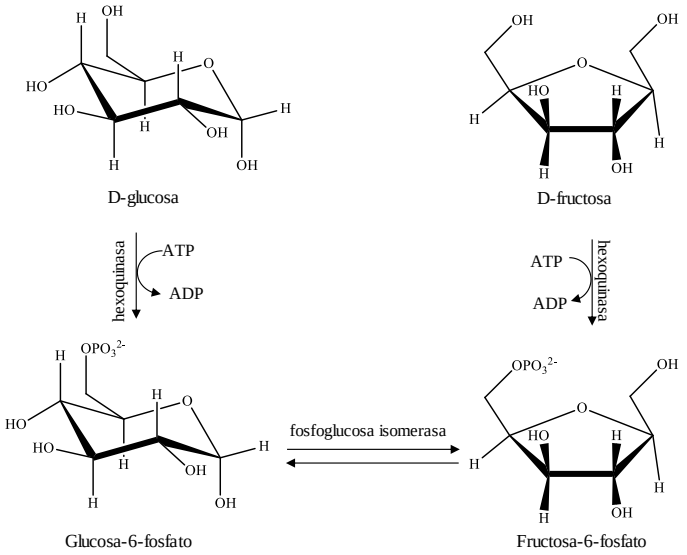


Figura 14. Fosforilación de glucosa y fructosa, y conversión de glucosa 6-fosfato a fructosa 6-fosfato.

Fosforilación de la fructosa 6-fosfato a fructosa 1,6-difosfato

Se considera la segunda reacción de activación de la glucólisis, y consiste en la transferencia de un grupo fosforilo desde el ATP a la fructosa 6-fosfato, para dar fructosa 1,6-difosfato (Figura 15). Esta reacción irreversible está catalizada por la enzima fosfofructoquinasa-1, denominada así para distinguirla de su homónima fosfructoquinasa-2 que catalizaría la formación de fructosa 2,6-difosfato en otra ruta metabólica.

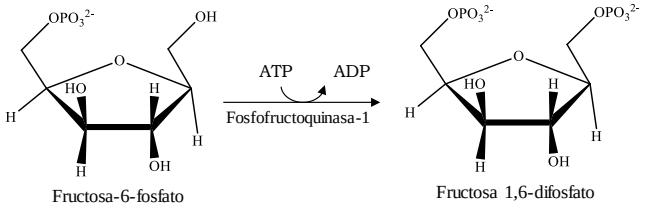


Figura 15. Fosforilación de la fructosa 6-fosfato a fructosa 1,6-difosfato

Ruptura de la fructosa 1,6-difosfato

Este paso consiste en la ruptura de la fructosa 1,6-difosfato para dar dos triosas, dihidroxiacetona fosfato y gliceraldehído 3-fosfato (Figura 16). Esta reacción es una condensación aldólica reversible catalizada por la enzima fructosa 1,6-difosfato aldolasa, o también conocida simplemente como aldolasa.

Interconversión de las triosas fosfato

De las dos triosas formadas, únicamente puede continuar la ruta metabólica el gliceraldehído 3-fosfato. Por ello, inmediatamente la dihidroxiacetona fosfato es convertida a gliceraldehído 3-fosfato (Figura 16.) en una reacción reversible catalizada por la enzima triosa fosfato isomerasa

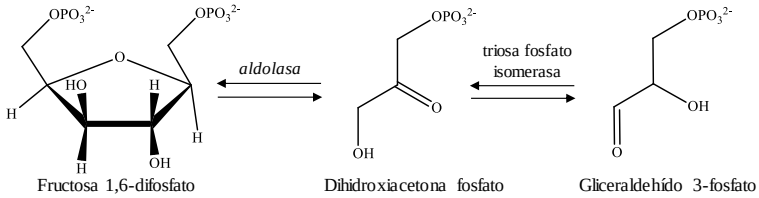


Figura 16. Ruptura de la fructosa 1,6-difosfato

Oxidación del gliceraldehído 3-fosfato a 1,3-disfosfoglicerato

Este paso consiste en la conversión de gliceraldehído 3-fosfato a 1,3-disfosfoglicerato (Figura 17), catalizada por la enzima gliceraldehído 3-fosfato deshidrogenasa. Donde el grupo aldehído del gliceraldehído 3-fosfato es oxidado, pero no a un grupo carboxilo libre sino a un grupo fosfato.

Transferencia de fosforilo desde el 1,3-difosfoglicerato al ADP

Del 1,3-difosfoglicerato se transfiere un grupo fosforilo al ADP, en una reacción catalizada por la enzima fosfoglicerato quinasa, obteniéndose 3- fosfoglicerato y una molécula de ATP (Figura 17).

Conversión del 3-fosfoglicerato en 2-fosfoglicerato

En este paso se realiza un desplazamiento del grupo fosforilo del carbono C3 del 3-fosfoglicerato al carbono C2 para formar el 2-fosfoglicerato (Figura 17). Esta reacción es reversible y está catalizada por la enzima fosfoglicerato mutasa.

Deshidratación del 2-fosfoglicerato a fosfoenolpiruvato

La enzima enolasa cataliza la reacción reversible de la pérdida de una molécula de agua desde el compuesto 2-fosfoglicerato para formar el fosfoenolpiruvato (figura 17).

Transferencia de fosforilo desde el fosfoenolpiruvato al ADP

Este sería el último paso de la ruta metabólica, que consiste en la transferencia de un grupo fosforilo desde el fosfoenolpiruvato al ADP, reacción catalizada por la enzima piruvato quinasa (Figura 17). Además de una molécula de ATP, se produce piruvato en su forma enol, y a continuación se tautomeriza no enzimáticamente a la forma ceto, que es la más abundante a pH 7.

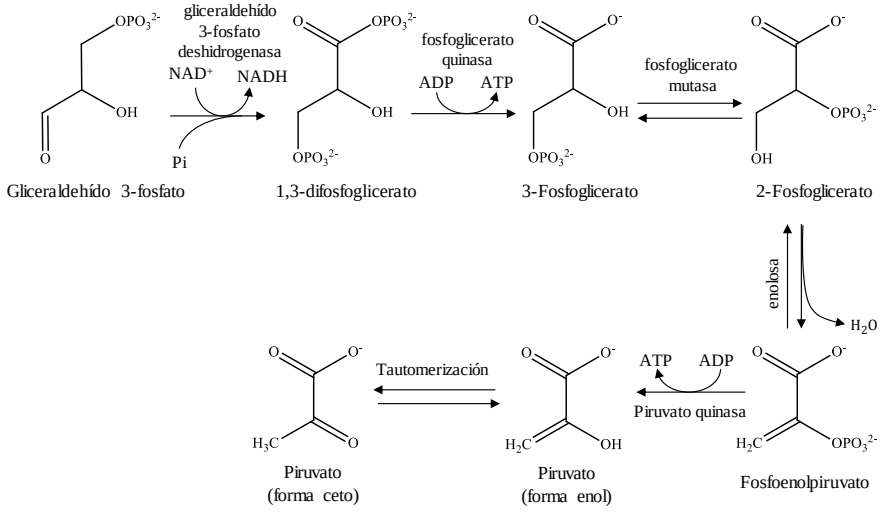


Figura 17. Formación del piruvato a partir de gliceraldehído 3-fosfato.

El piruvato es un compuesto muy reactivo que dependiendo de las condiciones puede derivar en multitud de compuestos. En el caso de la fermentación alcohólica el piruvato se transforma en etanol (Figura 18), en dos reacciones catalizadas enzimáticamente.

La primera reacción es la descarboxilación del piruvato, dando lugar a una molécula de CO_2 y una de acetaldehído, reacción catalizada por la enzima piruvato descarboxilasa. En la segunda reacción tiene lugar la reducción del acetaldehído para dar etanol, gracias al poder reductor del NADH obtenido de la deshidrogenación de gliceraldehído 3-fosfato, está reacción está catalizada por la enzima alcohol deshidrogenasa.

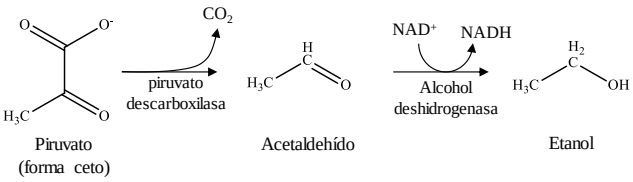


Figura 18. Formación de etanol a partir de piruvato.

Como ya hemos comentado, durante la fermentación alcohólica se produce etanol y CO_2, quedando la siguiente ecuación global de la ruta metabólica:

$$C_6H_{12}O_6+2ADP+2H_3PO_4 \longrightarrow 2CH_3CH_2OH+2CO_2+2ATP+2H_2O$$

4.3. Reacciones secundarias

El etanol no es el único producto que se obtiene durante la fermentación alcohólica, se pueden dar reacciones secundarias que le aportan diferentes cualidades al vino resultante, ya sean deseables o no.

El ácido succínico y la glicerina serían los compuestos secundarios más abundantes que se dan en la fermentación alcohólica (Torija *et al.*, 2003b), pero se pueden encontrar otros como el ácido acético, el ácido láctico, el ácido propiónico, entre otros (Fan *et al.*, 2014).

El ácido succínico se puede formar tanto en condiciones de anaerobiosis, como de aerobiosis. En condiciones de anaerobiosis el piruvato es transformado en ácido oxalacético en una reacción donde se incorpora anhídrido carbónico, catalizada por la enzima piruvato carboxilasa; el ácido

oxalacético se reduce a ácido málico con la ayuda del poder reductor del $NADH_2$; el ácido fumárico se forma por la deshidratación del ácido málico; y por último el ácido succínico se forma por la reducción del ácido fumárico con la ayuda de una molécula de $NADH_2$ (Figura 19).

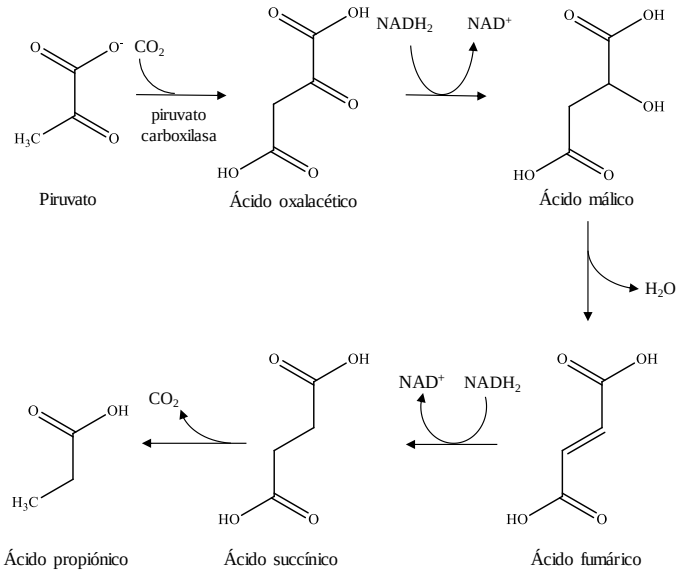


Figura 19. Formación de ácido succínico en condiciones de anaerobiosis

En condiciones de aerobiosis el piruvato es transformado a acetil-CoA, mediante una reacción catalizada por la enzima piruvato deshidrogenasa, y este se incorpora al ciclo de Krebs (Figura 20). Donde el α-cetoglutarato se oxida a succinil-CoA, catalizado por la enzima α-cetoglutarato deshidrogenasa y de nuevo se vuelve a oxidar con la intervención de la enzima succinil-CoA sintetasa para dar finalmente ácido succínico.

El glicerol o glicerina es uno de los compuestos secundarios más abundante de la fermentación y se puede formar en el proceso conocido como fermentación gliceropirúvica (Figura 21). En él, la dihidroxiacetona formada durante la glucólisis puede reducirse a glicerol 3-fosfato en una reacción catalizada por la enzima glicerol fosfato deshidrogenasa. El glicerol se obtendrá en una segunda reacción de hidrólisis del glicerol 3-fosfato, catalizada por la enzima glicerol 3-fosfato fosfatasa.

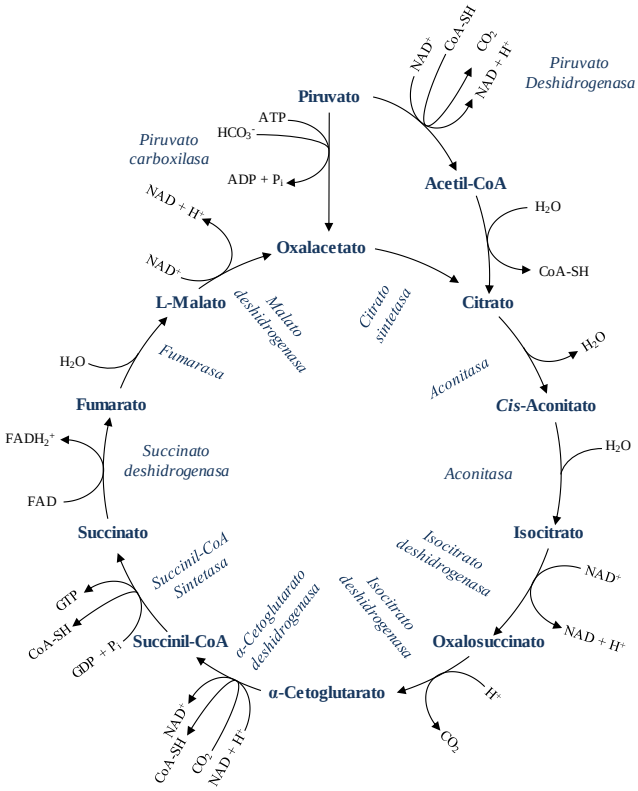


Figura 20. Formación de ácido succínico en condiciones de aerobiosis, ciclo de Krebs.

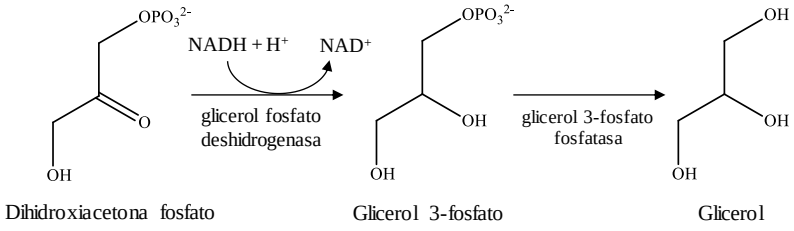


Figura 21. Fermentación gliceropirúvica.

Otros compuestos secundarios menos abundantes pero que también pueden modificar las cualidades del vino son el ácido propiónico, que como se puede ver en la Figura 19 se puede formar a partir del ácido succínico. El ácido láctico se obtiene de la fermentación láctica, que consiste en la reducción del piruvato a lactato (Figura 22), gracias al poder reductor del $NADH_2$.

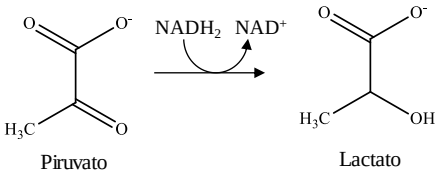


Figura 22. Fermentación láctica.

Uno de los compuestos secundarios más indeseables en la fermentación alcohólica es el ácido acético, que le otorga al vino sabores y olores desagradables, conocidos como picado. El ácido acético se puede formar en condiciones de anaerobiosis por dos vías (Figura 23): La primera vía es la descarboxilación catalizada por la enzima piruvato descarboxilasa del piruvato para dar acetaldehído, donde este aldehído se oxidará a acetato con la enzima aldehído deshidrogenasa; en la segunda vía el piruvato en primer lugar se oxida y descarboxila catalizado por la piruvato deshidrogensa formando acetil-CoA, y este derivará en acetato con la actividad de la enzima acetil-CoA hidrolasa.

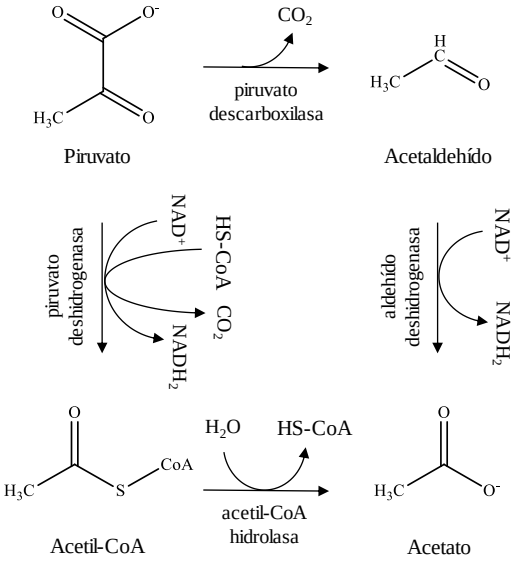


Figura 23. Formación de ácido acético en condiciones de anaerobiosis

4.4. Factores determinantes de la fermentación

La fermentación como ya se ha comentado anteriormente es un proceso complejo que conlleva numerosas reacciones catalizadas enzimáticamente, pero además presenta numerosos factores que son determinantes en el desarrollo de dicha fermentación, así como para la calidad del vino final. Algunos de los factores más importantes son la temperatura, la aireación, el pH, la clarificación, la presión osmótica y los nutrientes.

4.4.1. Temperatura de fermentación

La temperatura de fermentación es un factor muy importante para el desarrollo de las levaduras, donde una temperatura muy alta o muy baja puede derivar en fermentaciones lentas o en paradas de fermentación. En los vinos blancos se suelen utilizar temperaturas entre 10 – 15 ºC, para favorecer la producción y la retención de compuestos volátiles y así mejorar el perfil aromático del vino (Sablayrolles, 2009; Torija *et al.*, 2003a). En cambio, en la vinificación en tintos no se presta tanta atención a los compuestos volátiles, pero si a la transferencia de compuestos fenólicos desde las partes sólidas a la fase líquida (Sablayrolles, 2009). Esta transferencia se ve favorecida con temperaturas más altas y un alto contenido en etanol, por ello los vinos tintos se pueden fermentar hasta 30 ºC (Sacchi *et al.*, 2005). En el caso de los arándanos, autores como Yan *et al.* demostraron que la temperatura óptima de fermentación era 22.65 ºC (Yan *et al.*, 2012).

Un aumento de la temperatura puede provocar una mejora en la cinética de la fermentación, pero también un cambio en el metabolismo de la levadura. Esto puede derivar en un menor rendimiento en etanol, no solo por la evaporación de este, sino por el aumento de productos de vías secundarias, como puede ser el glicerol y el ácido acético, entre otros (Torija *et al.*, 2003b)

4.4.2. Aireación

La aireación es un proceso importante en la fermentación, puesto que el oxígeno es necesario para las levaduras. Las levaduras son anaerobias facultativas, pueden desarrollar la fermentación en condiciones de anaerobiosis, pero a su vez necesitan oxígeno para llevar a cabo la respiración aerobia y aumentar su crecimiento. Pero, un alto contenido de oxígeno puede desembocar en una parada de la fermentación, conocida como efecto pasteur, puesto que las levaduras realizan la respiración aerobia y no la fermentación.

4.4.3. pH

El pH no tiene una influencia fundamental en el proceso de fermentación, pero, tiene efectos positivos como facilitar la hidrólisis de los disacáridos a un pH bajo; y efectos negativos como incrementar el efecto tóxico del etanol y del sulfito. El pH óptimo durante la fermentación alcohólica para el crecimiento de las levaduras está en el rango de 3 a 5 (Ward, 1991).

4.4.4. Clarificación

La clarificación de un mosto es un factor importante para tener en cuenta en el proceso fermentativo, puesto que, si no está bien clarificado y presenta muchas partículas, estas pueden favorecer la formación de burbujas de CO_2, favoreciendo su disipación. En cambio, si los mostos están demasiado clarificados pueden tener una merma en nutrientes que son esenciales para el crecimiento de las levaduras (Moio *et al.*, 2004), por ejemplo, los nutrientes nitrogenados.

4.4.5. Presión osmótica

La presión osmótica ejercida sobre las levaduras es vital para su supervivencia, puesto que un mosto con un alto contenido en azúcar puede provocar la muerte de las células por la salida del agua citoplasmática a través de la membrana. Las levaduras que se usan normalmente en la industria tienen una limitada osmotolerancia (Kiran Sree *et al.*, 2000), pero autores como García-Martínez *et al.* aislaron cepas de levaduras osmotolerantes de mostos parcialmente fermentados de uva Pedro Ximenez (García-Martínez *et al.*, 2011).

4.4.6. Nutrientes

Las levaduras son seres vivos unicelulares que necesitan nutrientes orgánicos y minerales para llevar a cabo su metabolismo. El nitrógeno es un macronutriente, que juega el papel más importante en el proceso fermentativo (Bell and Henschke, 2005). Las levaduras necesitan nitrógeno asimilable para la síntesis de las proteínas para su crecimiento, y lo pueden tomar del ion amonio, de los aminoácidos y de los péptidos de bajo peso molecular.

5. VINOS DE FRUTAS

Según la OIV, el vino se puede definir como: *"exclusivamente la bebida resultante de la fermentación alcohólica completa o parcial de la uva fresca, estrujada o no, o del mosto de uva. Su graduación alcohólica adquirida no puede ser inferior a 8.5% vol".*

Esta organización establece que el vino solo puede ser obtenido a partir de la uva y no de otras frutas. Lo mismo ocurre en el caso de la sidra, que según el Real Decreto 72/2017, del 10 de febrero se puede definir como: *"Producto resultante de la fermentación total o parcial del mosto de manzana, al que se puede incorporar, posteriormente a la fermentación, los azúcares o jarabes azucarados, regulados en la normativa sobre determinados azúcares destinados a la alimentación humana, y anhídrido carbónico. Su grado alcohólico volumétrico adquirido será igual o superior a 4% vol".*

En cambio, en la definición de vino de la Real Academia Española, si se acepta el uso de todo tipo de frutas para la elaboración de un vino, y que pueda recibir dicho nombre.

"1. Bebida alcohólica que se hace del zumo de las uvas exprimido, y cocido naturalmente por la fermentación.
2. Zumo de otras plantas o frutos que se cuece y fermenta al modo del de las uvas."

Además, la Asociación de Productores de Sidra y Vinos de Frutas de la Comunidad Económica Europea (CEE) definen los vinos de frutas como: *"bebida alcohólica obtenida por la fermentación parcial o completa de jugos de frutas frescos, jugo concentrado o reconstituido; o macerado de pulpa con la adición de agua, azúcar o miel. Finalizada la fermentación se puede adicionar jugo fresco, concentrado o reconstituido".*

Es por este motivo que, durante la redacción de esta tesis doctoral, se han utilizado indiferentemente los términos vino de arándanos y bebida fermentada de arándanos.

Entre todas las frutas, la uva es la más utilizada en la producción de vino, seguido de la manzana en la elaboración de vino de manzana o sidra (Wei *et al.*, 2019). Sin embargo, se podría obtener vino a partir de toda aquella planta o fruta que pueda ser fermentada en un proceso igual o similar al de la vinificación de la uva o la manzana. En países como Hawái, Nigeria, Japón y Colombia se elabora vino de piña, en Dinamarca vino de cereza, en Japón, Corea y China se elabora vino de ciruela, en Missouri vino de tomate, en Florida vino de aguacate y arándano, en Nueva York vino de melón, en Oregón vino de tila, en Minnesota vino de sandía, en Colombia vino de mango, guayaba y mora, en la India el vino de kiwi… En España se elaboran diferentes tipos de vinos de frutas como el vino de naranja, en la provincia de córdoba o el vino de plátano, en las islas canarias. Existen muchos tipos de frutas que se pueden destinar a la vinificación y se ha investigado elaborando vinos de kiwi, pera, naranja, arándano, cereza, melocotón, frambuesa, entre otras (Jagtap and Bapat, 2015; Wei *et al.*, 2019).

El vino de arándano puede tener su origen hace siglos en los aquelarres de brujas, puesto que es un fruto que se utilizaba en los ritos de magia negra, para elaborar licores con un alto contenido de alcohol que permitía a los participantes entrar en un profundo trance. El primer vino de arándanos comercial se elaboró en México en 2013 bajo la marca *"Berry Me"*, donde se destinaba un 70 % de su producción a la exportación a Estados Unidos, donde tuvo una rápida aceptación por el consumidor, mientras que el 30 % restante se comercializa en el distrito federal. Actualmente, en Chile se produce vino de arándano y vino de arándano espumoso. En Florida, se produce también vino de arándano e incluso vino dulce de arándano.

En los últimos años el vino de arándano ha sido objeto de estudio de numerosos autores, donde se ha optimizado las condiciones de fermentación, temperatura (22.65 ºC), pH (3.53) y tamaño del inóculo (7.37 %), para obtener el máximo contenido alcohólico y la mínima acidez volátil (Yan *et al.*, 2012); se ha analizado su capacidad antioxidante, el contenido en compuestos fenólicos y sus propiedades organolépticas (Fu *et al.*, 2015; Johnson *et al.*, 2013; Johnson and Gonzalez de Mejia, 2012; Ortiz *et al.*, 2013; Rupasinghe and Clegg, 2007; X. Sun *et al.*, 2019). Se ha estudiado la influencia de la técnica de maceración enzimática a baja temperatura sobre la composición en

compuestos fenólicos (Gao *et al.*, 2015). Además, algunos autores han estudiado la vinificación de los arándanos con otra frutas como la banana (Seo *et al.*, 2015) o la mora (Johnson *et al.*, 2013).

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CAPÍTULO II

HIPÓTESIS Y OBJETIVOS

HIPÓTESIS Y OBJETIVOS

A continuación, se van a desarrollar las hipótesis y los objetivos que se han abordado en esta Tesis Doctoral.

Hipótesis 1

Los arándanos son alimentos funcionales debido a las propiedades beneficiosas que presentan para la salud, entre las que pueden destacar anticancerígena, antioxidante y antiinflamatoria, entre otras. Estas propiedades son consecuencia de un alto contenido en compuestos fenólicos. Por este motivo, en los últimos años se ha estudiado la vinificación del zumo de estos frutos, pero uno de los principales problemas que se encuentran es la baja graduación alcohólica que se alcanza, y la necesidad de adicionar disoluciones azucaradas o, directamente, azúcar al mosto.

Objetivo 1

El objetivo fue estudiar la deshidratación de los arándanos como método de aumento del azúcar en el mosto. Además, se evaluaron los cambios en los compuestos bioactivos, color y actividad antioxidante durante el secado y la vinificación de los arándanos, así como, la aceptación en el consumidor de los vinos con diferente grado de azúcar residual. Los resultados obtenidos se encuentran recogidos en el Capítulo III, en el artículo **"Bioactive compounds of chamber-dried blueberries atcontrolled temperature and wines obtained from them"**.

Hipótesis 2

Desde el punto de vista enológico, el secado de las uvas es muy importante. En el sur de España, es una técnica muy utilizada en la elaboración de sus vinos dulces. El proceso de secado de frutas ha sido ampliamente utilizado en la conservación de frutas. Tradicionalmente, se utilizaban las condiciones ambientales para obtener un producto seco, pero se ha comprobado que presentan numerosas desventajas. Por el contrario, los métodos de secado en cámara con aire caliente a temperatura contralada son fiables y fáciles de usar.

Actualmente, se está estudiando el aumento del rendimiento del secado convectivo en cámara empleando metodologías de tratamiento previas al

secado, como puede ser la congelación o los tratamientos de inmersión en disoluciones alcalinas, entre otros; o el uso de este tipo de secado para obtener nuevos productos, como puede ser la deshidratación de los arándanos.

Objetivo 2

El objetivo fue mejorar el rendimiento en el secado de uva, tras la perforación de la baya desprovista de pedicelo, evaluar modelos predictivos para la cinética de secado de los frutos y comprobar la influencia de la temperatura y la perforación de la baya en el contenido de compuestos fenólicos y en la actividad antioxidante. Los resultados obtenidos se encuentran recogidos en el Capítulo IV, en el artículo **"The influence of berry perforation on grape drying kinetics and total phenolic compounds"**.

Objetivo 3

El objetivo fue evaluar el efecto que tiene la temperatura en las cinéticas de secado de arándanos, estudiando su ajuste a diferentes modelos matemáticos; y evaluar la importancia de la temperatura en el contenido de compuestos fenólicos, color y actividad antioxidante de los zumos resultantes. Los resultados obtenidos se encuentran recogidos en el Capítulo V, en el artículo **"Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (*Vaccinium corymbosum*)"**.

Hipótesis 3

La vinificación siempre se ha entendido como la elaboración de un vino a través del proceso de fermentación alcohólica de los mostos de la uva. Pero cada vez más, se está estudiando la elaboración de nuevos productos, como son los denominados vinos de fruta. En esta definición entran todas las bebidas obtenidas de la fermentación del zumo de una fruta, entre las que podemos encontrar la manzana (denominada sidra), el kiwi, la pera, la naranja, el arándano, la cereza, el melocotón y la frambuesa, entre otras.

Objetivo 4

El objetivo fue estudiar la fermentación de mostos de uva y arándano en una proporción 1:1 con diferentes cepas de levaduras, con el fin de evaluar el efecto de la levadura en el contenido de compuestos fenólicos, color y

actividad antioxidante del nuevo vino elaborado. Los resultados obtenidos se encuentran recogidos en el Capítulo VI, **"Phenolic compounds, antioxidant activity and color in the fermentation of blueberry and grape juice with different yeasts"**.

Objetivo 5

El objetivo era estudiar la vinificación de mostos de uvas deshidratadas y arándanos frescos y de mostos de arándanos deshidratados y uvas frescas, ambos con proporción 1:1, con el fin de obtener un producto nuevo comercializable. Para ello se utilizarán las temperaturas y el tiempo de secado optimizadas en los capítulos IV y V; y las levaduras que menos compuestos bioactivos retengan, objeto de estudio en el capítulo VI. Los resultados obtenidos se encuentran recogidos en el Capítulo VII, **"Fermentation of blueberry and grape dried mix juices. Color, phenolics compounds and antioxidant activity"**.

CAPÍTULO III

RESULTADOS Y DISCUSIÓN

Bioactive compounds of chamber-dried blueberries at controlled temperature and wines obtained from them

ÍNDICE

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Bioactive compounds of chamber-dried blueberries at controlled temperature and wines obtained from them

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ABSTRACT

In this work, the effects of chamber drying under controlled temperature and moisture conditions and fermentation process on blueberry juices to obtain three wines were studied. The drying was made with a view to increase the sugar content and obtain wines with an ethanol content similar to a commercial grape wine or to obtain sweet wines. Analyses included color parameters, browning index and anthocyanin, flavonols, flavan-3-ol derivatives and tannin concentrations, as well as vitamin C concentration and antioxidant activity. Based on the results, drying increases color and the concentration of anthocyanins and tannins most probably by the effect of dehydration of the berries and diffusion of the colored compounds from the skin to the pulp due to the structural alterations in their skin. In addition, drying decreased flavonols, flavan-3-ol derivatives and vitamin C concentrations. The browning index, anthocyanins and tannins decreased with the fermentation time, and vitamin C was constant with the fermentation time. The sensory analysis showed that the wines with the best sensory characteristics were those with residual sugar, partial fermented wines 1 and 2.

KEYWORDS

Blueberry, drying process, fermentation, color, bioactive compounds

1. INTRODUCTION

Blueberries, *Vaccinium corymbosum*, are native to eastern North America and southeastern Canada (Williamson and Lyrene, 2004), however they are extending to Europe, Asia, Africa and Australia. The blueberries can be consumed as fresh fruits, but also in products derived as seasoning product of bakery, pastry filling, juices, yogurt, etc. (Routray and Orsat, 2011). Besides, in the last years there has spread the use of different berries for the elaboration of fermented beverages or wines, principally due to the fact that grapes are not well cultivated in every regions of the world. Another type of fruits is used for make the fermentation process, being the berries of red fruits some of the most used due to their phenolic composition, especially in anthocyanins content (Arozarena *et al.*, 2012). Yan *et al.*(Yan *et al.*, 2012) have studied the optimal conditions of the blueberries juice fermentation, being these conditions a temperature of 22.65ºC and a pH of 3.53. They carried out the fermentation of the blueberry juice adding sucrose, to reach the alcoholic graduation of a standard wine. Nevertheless, other authors have developed directly the fermentation on the juice, reaching only 5-6% of alcohol, due to the concentration of sugar (Johnson *et al.*, 2013; Su and Chien, 2007).

Blueberries are functional food due to their health benefits such as antioxidant, anti-inflammation, neuro-protection, anti-metastatic, cardio-protective, antimicrobial, reno-protective, opthalmoprotective, anti-diabetics, hepato-protective, gastro-protective, anti-osteoporotic, anti-aging (Patel, 2014). These properties are consequent of their phenolic composition (De Souza *et al.*, 2014). The protective activity is attributed to their antioxidant capacity and free radical scavenger in addition to the ability to inhibit and reduce enzymes (Li *et al.*, 2014). The phenolic compounds of blueberries include anthocyanins (anthocyanidins glycosides) which are in the cell vacuoles in the red fruit skins (Castañeda-Ovando *et al.*, 2009). They are responsible for the fruit color, between red and blue characteristic in blueberry. Flavonols are a flavonoid family ranging from white to yellow in color depending on their structure, but in red wines they can participate in the color due to they are copigments of the anthocyanins (Roger Boulton, 2001). Flavan-3-ol

derivatives are flavonoids compounds with optical isomerism, (+)-catechin and (-)-epicatechin are the most representative, and they are found in the seeds and stems of the grapes (Mateus *et al.*, 2001). Tannins encompassing a series of phenolic compounds including condensed tannins (viz. variably complex polymers of flavan-3-ol) and hydrolysable tannins (ellagitannins and gallotannins, mainly), they are highly astringent and bitter compounds present in seed of fruits.

The addition of sucrose is the most common method to increase the sugar content of blueberry juices (Fu *et al.*, 2015; Santos *et al.*, 2016; Zhang *et al.*, 2016) to obtain a fermented beverage with a standard ethanol content. Nevertheless, the partial dehydration of the berry can increase the sugar content in a similar way to that made in the southern Spain or Italy for the production of sweet wines. Sun-drying is the most traditional drying method, but artificial drying with controlled temperature, relative humidity and air flow had some advantages (Serratosa *et al.*, 2008) as avoid the losses for the weather (rains), or the attack of insects and fungal. These methods affect the berry properties such us color, texture and density (Krokida *et al.*, 1998).

The aim of this work was evaluating the changes in bioactive compounds, color and antioxidant activity during the drying process and subsequent fermentation of sugar-enrich juices to obtain blueberry wines.

2. MATERIAL AND METHODS

2.1. Reagents

Hydrochloric acid, metaphosphoric acid, formic acid, acetic acid, methanol, acetonitrile, sodium metabisulphite, potassium chloride, sodium acetate, acid potassium dichromate, ethyl acetate and potassium dihydrogen phosphate were purchased from Merck (Madrid, Spain). Anthocyanins (malvidin-3-O-galactoside chloride), flavan-3-ol derivative ((+)-catechin, (-)-epicatechin, epigalocatechin galate, procyanindin B1, procyanindin B2), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2,-diphenylpicrylhydrazyl) and DTT (DL-dithiothreitol) were purchased from Sigma-Aldrich Chemical Co. (Madrid, Spain).

2.2. Blueberry drying

The blueberries used for this study were Star variety (Vaccinium corymbosum) and they were harvested in 2015 in Moguer (Huelva, Spain) at the usual state of maturity for his commercialization. One portion were crushed for analysis.

Three batches of blueberries were dried in a Frisol Climatronic chamber at a constant temperature of 40ºC and an initial relative humidity of 20%. During the drying process, a sample was daily collected and the weight loss and reducing sugar concentration of the blueberries were measured using a refractometer model Atago Master.

The drying process was finished when the reducing sugar content reached approximately 24.2 ºBrix. The blueberries were pressed on a vertical press similar to industrial models, obtaining the final juice. The maximum pressure reached in each two pressing cycle was 300 bar. The musts were centrifuged at 5000 rpm, filtered and analyzed in triplicate.

2.3. Fermentation process

The sweet juice was divided into three batches and was mixed with the solid parts of the berries in a ratio 1:1 (w/v). The mix was added with a yeast inoculum of commercial Saccharomyces cerevisiae in a dose recommended by the manufacturer (0.3 g/L). The flasks were immersed in thermostatized water baths at 22 ± 0.5 °C (Yan *et al.*, 2012). Three wines were elaborated in triplicate from the dried blueberries.

Wine 1: The mix was partial fermented to 6% of ethanol (v/v) and the process was stopped by the addition of wine alcohol up to 17% (v/v).

Wine 2: The mix was partial fermented to 14% of ethanol (v/v) and the process was stopped by the addition of wine alcohol up to 17% (v/v).

Wine 3: The mixture was completely fermented obtaining a wine with a 17% (v/v) of ethanol content.

After fermentation/maceration, the berries were pressed a second time on a vertical press and skin residues removed from the wine. The resulting wines were centrifuged at 5000 rpm, filtered and analyzed in triplicate.

2.4. Reducing sugars

This parameter was determined according to the EEC official methods as described in Regulation 2676/1990 (*European Community, Official Journal of October 1990*, 1990).

2.5. Volatile acidity

Isolation of volatile acids is carried out according to the method of the OIV (*OIV, Collection of international methods of analysis of wines and musts*, 2005) by steam distillation and rectification of the distillate.

2.6. Spectrophotometric determinations

Spectrophotometric measurements were made on a PerkinElmer (Waltham, MA) Lambda 25 spectrophotometer, using quartz cells of 1 mm light path. Samples were previously passed through Millipore (Billerica, MA) HA filters of 0.45 µm pore size. All measurements were corrected for a path length of 1 cm.

Absorbances at 420, 520 and 620 nm were measured. Hue indicates the proportion between orange and red colors, A420 and A520 nm relation was calculated.

2.6.1. Polymeric pigments color (PPC)

PPC was obtained as the absorbance at 520 nm after 45 min of 5 mL of wine previously supplied with 15 mg of $Na_2S_2O_5$. Anthocyanin monomers were immediately decolorized by the excess $Na_2S_2O_5$ added, so the residual color was due to the polymeric forms of the pigments.

2.6.2. Antioxidant activity

Antioxidant activity was analyzed through the DPPH assay according to Alen-Ruiz *et al.* (Alén-Ruiz *et al.*, 2009) with some modifications. The juice and wines were diluted 1:5 with distilled water. A 45 mg/L solution of DPPH (2,2-diphenylpicrylhydrazyl) in methanol was prepared on a daily basis and stored in the dark. An 80 mg/L solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a vitamin E analogue was used as a standard. The analytical procedure was as follows: a 200 µL aliquot of diluted sample was placed in a cell and 3 mL of a 45 mg/L solution of DPPH in

methanol was then added. A blank (200 µL diluted sample + 3 mL methanol), a control sample (200 µL of 12% ethanol in water + 3 mL of DPPH solution) and a Trolox standard (200 µL of Trolox solution + 3 mL of DPPH solution) were also prepared in parallel. Following vigorous stirring, the absorbances at 517 nm of the control sample and blank were measured in a PerkinElmer (Waltham, MA) Lambda 25 spectrophotometer. The sample and the Trolox standard were measured under identical conditions after 120 min of incubation at room temperature. The results were expressed in millimoles of Trolox per liter.

$$\text{Antioxidant activity (mmol TE/L)} = \frac{(0.32 \cdot A_1 \cdot \text{dilution factor})}{A_2}$$

$$A_1 = \text{Absorbance}_{\text{control (t=0)}} - \text{Absorbance}_{\text{sample}}$$

$$A_2 = \text{Absorbance control}_{\text{(t=0)}} - \text{Absorbance standard}_{\text{(t=120)}}$$

$$\text{Absorbancia}_{\text{sample}} = \text{Absorbancia sample (t=120)} - \text{Absorbancia blanc (t=0)}$$

2.6.3. Total anthocyanins

The total monomeric anthocyanin pigment content was measured by pH differential method described by Lee *et al.* (Lee *et al.*, 2005). This method is based on the change of maximum of absorbance with a change in pH of the monomeric anthocyanins. Two dilutions 1:10 of juice samples was prepared with pH 1.0 buffer (potassium chloride) and pH 4.5 buffer (sodium acetate) and the absorbance was measured at 500 and 720 nm within 20-50 minutes of preparation. Calculate total anthocyanin concentration, expressed as cyanidin-3-glucoside equivalents, as follows:

$$\text{Total monomeric anthocyanins (mg/L)} = \frac{A \cdot M_W \cdot D \cdot 1000}{\varepsilon \cdot PL}$$

$$A = (A_{520} - A_{700})_{pH1} - (A_{520} - A_{700})_{pH4,5}$$

Where Mw is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), D is the dilution factor, ε is molar extinction coefficient for cyanidin-3-glucoside (26900 L/mol · cm) and PL is the light path.

2.6.4. Total tannins

The total tannins were determined by measurement of the absorbance at 550 nm after acid hydrolysis of the samples diluted 1:50 with distilled water and a

blank. The resulted absorbance (Asample-Ablank) was multiplied by a factor of 19.33, in order to calculate the total tannin concentration, in g/L.

2.6.5. Ethanol content

Ethanol content was determined according to Crowell *et al.* (Crowell and Ough, 1979), to this end, ethanol in the sample was collected by steam and then reacted with acid potassium dichromate. The reaction was spectrophotometrically monitored via the absorbance at 600 nm against a blank on a PerkinElmer Lambda 25 spectrophotometer.

2.7. Flavonols extraction

A volume of 2 mL of must was passed through a Sep-Pak C18 cartridge, with 900 mg of filling (Long Body Sep-Pak Plus; Waters Associates; Milford, Massachusetts) that was previously activated with 5 mL of pure methanol and washed with aqueous 0.01 % (v/v) HCl. The cartridge was successively washed with 10 mL of 0.01 % aqueous HCl. The cartridge was eluted with 5 mL of pure ethyl acetate. This collected fraction was evaporated on a rotary evaporator thermostated at 35 ºC and resolved in 1 mL of pure methanol. The fraction was passed through a filter of 0.45 µm pore size before injection into HPLC instrument.

2.8. Identification and quantification by HPLC-DAD of flavonols

A volume of 50 µL of the sample were injected into a HPLC-DAD instrument (Beckman Coulter System Gold, 168 Detector) and the analyses were carried out on a LiChrospher 100 RP-18 column (250 mm x 4.6 mm, 5 µm), according with the method proposed by Marquez *et al.* (Marquez *et al.*, 2012). The identification was carried out by comparing their retention times with those for standards, recording UV-vis spectra and calculating the UV absorbance ratios for samples and standards simultaneously co-injected one at a time.

2.9. Identification and quantification of flavan-3-ol derivatives

In the case of identification and quantification of flavan-3-ol derivatives, the samples were dilute 25 times in ultrapure water. The identification and quantification were carried out in a HPLC (Thermo Spectra Physic Series

P100) with fluorescence detector (PerkinElmer Series 200a), on a LiChrospher 100 RP-18 column (250 mm x 4.6 mm, 5 µm), according with the method proposed by Marquez *et al.* (Marquez *et al.*, 2012).

2.10. Vitamin C

0.7 mL of 4.5 % metaphosphoric acid was added to 0.7 mL of juice, the mix was centrifuged at 5000 rpm 10 minutes at 4 ºC. 1 mL of the mix was added to 0.2 mL of DTT (DL-dithiothreitol) solution and the sample was kept in dark for 2 hours in order to reduce the dehydroascorbic acid to L-ascorbic acid. After complete conversion, the sample was filtered with 0.45 µm nylon filter pore size. Ascorbic acid quantification was performed on a HPLC chromatograph (Thermo Spectra Physics Series P100) coupled to a UV detector (Thermo Finnigan Spectra System UV2000), using a LiChrospher 100 RP-18 column (250 mm x 4.6 mm, 5 µm). KH2PO4 (0.2 M at pH = 2.3-2.4) was used as mobile phase with a flow of 1.0 mL/min for 15 minutes at λ=243 nm and an injection volume of 20 µL.

2.11. Sensory analysis of wines.

The wines were assessed for aroma, flavour and colour acceptability in accordance with ISO 8586-1:1993. The tasting room was kept at 20ºC and wines served in coded tasting glasses certified in accordance with ISO 3591:1977. The sixteen tasters were instructed in advance about their task and the rules to be followed and were given a scoring sheet. The evaluation of the quality wines was made according to ISO 4121:2003, with options of desirable (5-6), acceptable (3-4) and undesirable (1-2).

2.12. Statistical Procedures.

The results for all samples were subjected to analysis of variance at the 95.0% confidence level, in addition homogeneous groups were calculated in order to stablish significates differences between means. The software used was the Statgraphics Computer Package v. 5.0 from Statistical Graphics Corp.

3. RESULTS AND DISCUSSION

The first stage in the elaboration of blueberry wine was a drying process in order to increase the sugar content to obtain a fermented beverage with an

ethanol content similar to a grape wine. The blueberry variety presented a sugar content of 13.2 ºBrix. This content increased to 24.2 ºBrix after 48 hours of drying. During the drying process evaporation of water occurred, causing the concentration of other compounds besides the sugars. A sweet juice was obtained from the dried blueberries and this juice was subjected to a fermentation process obtaining three wines with different sugar content due to the different time of fermentation. Wine 1 was a sweet wine with 117 g/L of residual sugars, wine 2 was a semi-sweet wine (28.8 g/L of residual sugars) and wine 3 was a dry wine (0 g/L of residual sugars).

The volatile acidity was measured in order to control the quality of wines. This parameter represents the amount of volatile acids in the wines, acetic acid being the majority. The volatile acidity increased with the fermentation time with values of 5.4, 5.9 and 8.9 meq/L for wine 1, wine 2 and wine 3, respectively. The latter presented a value higher than the limit established in the Official State Bulletin (*Official State Bulletin, Order of July 13, 2010, No. 169,* 2010) for commercial grape wines.

Color is the first attribute perceived by the consumer in food. The absorbances at 420, 520 and 620 nm as well as the hue were measured and were expressed as absorbance units (a.u.). These three absorbances represent the contribution of browning, and red and blue compounds respectively. As can be seen in Figure 1, the absorbances increased in the drying process, so the color intensity of the juice from dried fruits was higher than the juice from fresh fruits. In relation to wines, the browning index (A420) decreased with the fermentation time, wine 3 presented the lowest value. The values of absorbances at 520 and 620 nm were the highest in wine 2, presented the wine 3 the lowest values of both absorbances. This may be a result of the adsorption of colored compounds by the cell walls of the yeasts (Caridi, 2007). The hue, expressed as the ratio between absorbances at 420 and 520 nm (Figure 1d), decreased in the drying process, although the values were always above value 1, which indicates greater contribution of the brown compounds. In wines, the wine 3 (total fermentation) was the beverage with the lowest value.

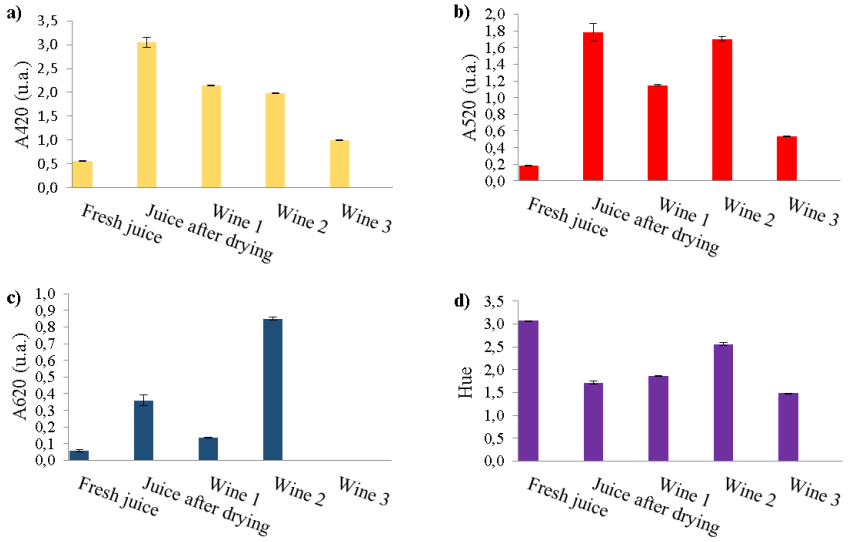

Figure 1. Changes in (a) absorbance at 420 nm (a.u.), (b) absorbance at 520 nm (a.u.), (c) absorbance at 620 nm (a.u.), (d) hue for blueberries juices and wines. N=3. Columns with different superscript letters are significantly different, p = 0.05.

The polymeric pigments color measured in wines shows that the fermentation process produced a decrease of PPC with the time (0.699, 0.517 and 0.373 to wine 1, 2 and 3 respectively), so the contribution of pigment polymers to color decrease. On one hand, the high value of wine 1 was due to the concentration as a result the loss of water by evaporation during the drying process. On the other hand, the maceration with solid parts should increase the values during the fermentation, nevertheless these compounds decreased.

Anthocyanins are polyphenolic pigments which are responsible of the red color of blueberry juices and wines. The drying process increased the concentration of these compounds due to the water evaporation and the diffusion process from skin to juice due to the structural alterations in fruit skin (Marquez *et al.*, 2013a). After the drying, the anthocyanins content was 40 mg/L (Figure 2). In wines the concentration of monomer anthocyanins decreased with the fermentation time, being wine 3 which presented the lower concentration. This fact could be due to, on the one hand, the adsorption of anthocyanin by the cell membranes of yeast, as the time of contact with yeasts increases, a reduction of these compounds occurs in the juice (Caridi, 2007). Furthermore, condensation reactions occur between the monomeric anthocyanins and some yeast metabolites resulting new compounds called

pyranoanthocyanins (Marquez *et al.*, 2013b) or condensation reactions with other phenolic compounds such as flavonols and flavans (He *et al.*, 2012).

Regarding the concentration of total vitamin C, fresh blueberries showed the highest value (28.1 mg/L) (Figure 2). The drying process produced a decrease in total vitamin C content to 21.5 mg/L. This vitamin C concentration was maintained without significant changes during the fermentation process. Vitamin C is a typical heat sensitive micronutrient, so the decrease during drying process is due to the temperature (Dhuique-Mayer *et al.*, 2007). Then, the fermentation process did not affect the content of this vitamin.

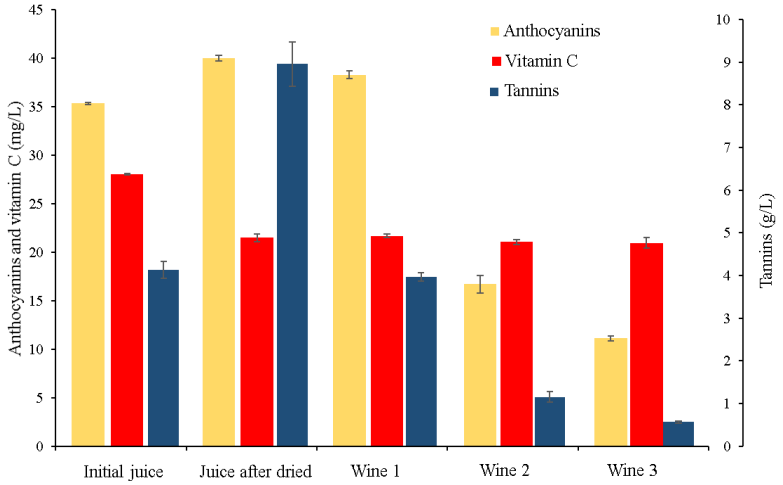

Figure 2. Changes in anthocyanins, vitamin C and tannins of blueberries juices and wines. N=3. Columns of the same parameter with different superscript letters are significantly different, p = 0.05.

Tannins are polymer compounds responsible for the astringency of wines. In wines a certain astringency is appreciable, although if it is excessive it becomes a defect. Drying process raised its content from 4.13 to 8.95 g/L (Figure 2), due mainly to the concentration effect by water evaporation. However, the fermentation process produced a decrease in total tannin values, which was more pronounced as the fermentation time increased. The final values of the wines were 3.97 g/L in wine 1 (less fermentation time) and 1.15 and 0.570 g/L for wine 2 and wine 3, respectively. This fact can be contrary in other fermentation process where tannin content increase by the effect of their synthesis and/or extraction in an ethanol medium. Tannins in grape seeds

are slowly dissolved during maceration as the cuticle is dissolved in ethanol; by contrast, tannins in grape skins were extracted more rapidly by the effect of their ready dissolution in the aqueous phase (Guadalupe and Ayestarán, 2008).

Table 1 shows the concentrations of flavonols and flavan-3-ol derivatives in blueberry juices and wines. Four flavonols were identified, two quercetin derivatives and two syringetin derivatives. Quercetin derivatives presented higher concentrations than those of syringetin in the juice from fresh fruit, in addition the galactose derivatives were found in higher concentration in both cases. The drying process decreased the concentration of this phenolic compounds. Some authors found drying process increase the concentration of flavonols (Marquez *et al.*, 2012). During the fermentation process occurs a maceration process with the berry skins, so these compounds should be extracted from skins, however the concentration decreased, this reduction can be ascribed to co-pigmentation reactions with anthocyanins (Roger Boulton, 2001). The final concentration in wines is a balance between the extraction from skins and the reactions with other compounds, obtaining wine 2 with the highest flavonol concentration.

Also, five flavan-3-ol derivatives were identified and quantified, 3 monomers and 2 procyanidins. The major compound in juice from fresh fruit was procyanidin B1 followed by catechin. Drying and fermentation stages caused a decrease in concentration of total flavan-3-ol derivatives, although procyanidin B1 increased during drying and epicatechin could be quantified. These compounds are involved in different reactions. Catechins and proanthocyanidins are the main substrates for condensation with monomeric anthocyanins and their subsequent evolution to polymeric anthocyanins (Budić-Leto *et al.*, 2006).

Table 1. Flavan-3-ol and flavonols concentration (mg/L) and homogenous groups for blueberries juices and wines at different fermentation times.

	Fresh juice	Juice after drying	Wine 1	Wine 2	Wine 3
Quercetin-3-O-galac	10.6±0.862^{a}	4.93±0.232^{b}	1.87±0.104^{c}	2.18±0.044^{c}	1.88±0.011^{c}
Quercetin-3-O-gluc	7.13±1.03^{a}	4.04±0.123^{b}	3.05±0.221^{c}	3.68±0.078^{bc}	3.08±0.028^{c}
Siringetin-3-O-galac	4.25±0.569^{a}	3.28±0.249^{b}	2.06±0.116^{d}	2.64±0.084^{c}	2.61±0.015^{c}
Siringetin-3-O-gluc	2.08±0.351^{a}	3.60±0.292^{b}	3.10±0.302^{c}	3.48±0.039^{bc}	3.22±0.160^{bc}
Total flavonols	24.1±0.382^{a}	15.8±0.897^{b}	10.1±0.106^{c}	12.0±0.247^{d}	10.8±0.139^{c}
Procyanidin B1	19.5±1.32^{a}	24.6±1.68^{b}	14.9±0.422^{c}	15.5±0.120^{c}	17.0±1.19^{a}
Epigallocatechin gallate	2.87±0.064^{a}	2.78±0.085^{a}	2.88±0.336^{a}	3.12±0.045^{a}	4.84±0.650^{b}
Catechin	15.9±1.19^{a}	5.38±0.526^{b}	4.22±0.776^{b}	4.95±0.282^{b}	4.51±0.557^{b}
Prociayanidin B2	3.17±0.622^{a}	2.80±0.187^{a}	3.45±0.673^{a}	3.37±0.087^{a}	3.59±0.609^{a}
Epicatechin	n.d.	2.20±0.136^{a}	3.06±0.134^{c}	2.26±0.057^{a}	3.87±0.542^{b}
Total flavan-3-ol	41.5±0.687^{a}	37.8±1.388^{b}	28.5±1.321^{c}	29.2±0.237^{c}	33.8±0.117^{b}

Figure 3 shows the values of antioxidant activity measured by DPPH assay in juices and wines from blueberries. As can be seen, the juice from fresh fruit had a high antioxidant activity, this value decreases after the drying process. The wines 1 and 2 obtained from the sweet juice had no significates differences, wine 3 (total fermented wine) presented the lowest value. Antioxidant activity has been related with phenolic compound and vitamin C. Some authors have found that the anthocyanins are the main responsible of the antioxidant activity of berry fruits, and the vitamin C affect less to this activity. Specifically, a linear relation between total anthocyanins and antioxidant activity has been found in blueberry varieties (Mi *et al.*, 2004) and others have found that vitamin did not contribute to the antioxidant activity values in blueberries (Borges *et al.*, 2010). Some authors state that the antioxidant activity is closely related to compounds of high molecular weight (Marquez *et al.*, 2014; Tlili *et al.*, 2015). In this work the antioxidant activity decreased during the drying process and during the fermentation and there was no significant correlation between antioxidant activity, measured by the DPPH assay, and the composition of phenolic compounds, although there was a statistically significant relationship between antioxidant activity and vitamin C at the 99% confidence level, since the p-value in the ANOVA analysis is less than 0.01.

Figure 4 shows the scores obtained in the sensory analysis of the three wines. Wine 1 presented a more balanced score, standing out on others in the aroma. This can be due to the fact that fermentation process was shorter, so varietal aromas were higher than in wine 2 and wine 3. Wine 2 also presented an acceptable score for the tasters, standing out on the others in the flavor, as result of the compensation between the acid flavor of the blueberry and the sweetness of a semi-sweet wine. On the other hand, in spite of having the worst score in flavor and aroma, wine 3 presented the best score in color, as consequence of the highest contact time with the solid parts during the fermentation.

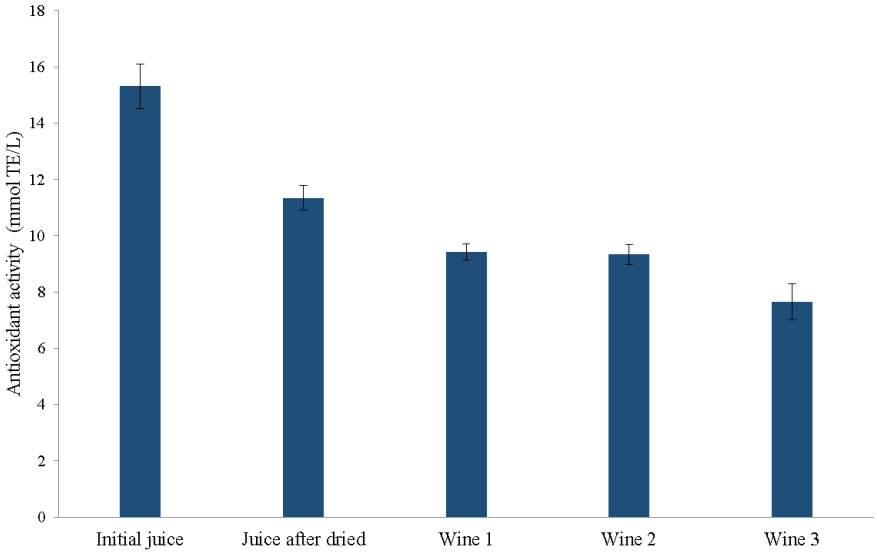

Figure 3. Antioxidant activity for blueberries juices and wines. N=3. Columns with different superscript letters are significantly different, p = 0.05.

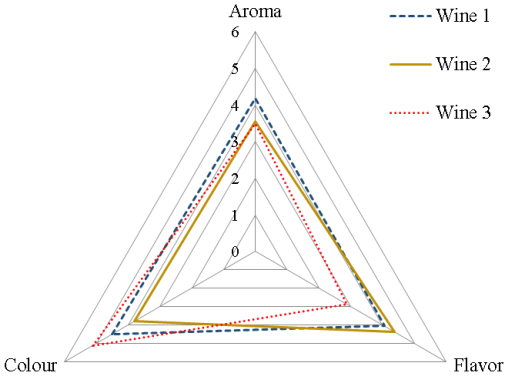

Figure 4. Changes in (a) absorbance at 420 nm (a.u.), (b) absorbance at 520 nm (a.u.), (c) absorbance at 620 nm (a.u.), (d) hue for blueberries juices and wines. N=3. Columns with different superscript letters are significantly different, p = 0.05.

4. CONCLUSIONS

The winemaking of blueberry wines caused changes in color and concentration in bioactive compounds. The drying process increased the absorbances at 420, 520 and 620 nm, so the browning index and the contribution of red compounds were higher in the juice after drying. The concentration of anthocyanins and tannins also increased with drying, however the flavonols, flavan-3-ol derivatives and vitamin C decreased in this stage. The fermentation stage caused a decrease in phenolic compounds, tannins and antioxidant activity, while concentration of vitamin C was constant. Wine 3 presented the less values of anthocyanins and tannins as well as antioxidant activity, in addition to a high volatile acidity. The sensory analysis showed that partial fermented wines had the best score. In this sense, in the production of blueberry wines, the drying process is suitable to obtain juice enriched in sugars, the partial fermentation obtains better results than total fermentation. Further study on drying stage as well as the fermentation could improve the characteristics of these wines.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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CAPÍTULO IV

RESULTADOS Y DISCUSIÓN

The influence of berry perforation on grape drying kinetic and total phenolic compounds

ÍNDICE

Journal of the Science of Food and Agriculture SCI

The influence of berry perforation on grape drying kinetic and total phenolic compounds

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ABSTRACT

BACKGROUND: Drying is one of the traditional methods used for the conservation of fruits. In recent years, different methods have been developed to obtain higher quality products. The chamber-drying methods with hot air at controlled temperature, are reliable and easy to use. The effect of piercing the structure of grape berries on their drying time was studied experimentally during convective drying within a temperature range of 30–50 ºC. Experimental moisture loss results were fitted to different mathematical models, evaluated for goodness of fit by comparing their respective R^2, χ^2, and RMSE.

RESULTS: The Midilli *et al.* model provided a better prediction to describe the drying of whole grapes. However, the punched grapes showed a better fit for the Two-term model at 30 and 40 ºC, and the approximation of diffusion model at 50 ºC. The values of effective moisture diffusivity fluctuated between 8.04 x 10^{-12} and 7.31 x 10^{-11} m^2/s. Activation energy was 56.49 and 54.43 kJ/mol for whole and punched grapes, respectively. All the drying processes produced an increase of total phenolic compounds and antioxidant activity in grapes, being these increases higher in the whole grape drying.

CONCLUSION: The drying of punched grapes was faster and the activation energy was higher than the drying of whole grapes, however the whole grapes presented more total phenolic compounds and antioxidant activity.

KEYWORDS

Grape, chamber-drying, kinetic models, total phenolic compounds, antioxidant activity

INTRODUCTION

Tempranillo is one of the most important grape varieties in Spain, being the main one of the Rioja Denomination of Origin, however, it has spread throughout the country. This variety is authorized in 38 denominations of origin of Spain and is considered as the preferential variety in 14 of them.

Berry red fruits, like grapes, are widely studied due to their composition in bioactive compounds (Aleixandre-Tudo *et al.*, 2017; Borochov-Neori *et al.*, 2015; Skrovankova *et al.*, 2015), they have antioxidant properties, and protect cells from damage caused by reactive oxygen species. Cell damage is caused by oxidative stress, due to an imbalance between antioxidants and reactive oxygen species, related to cancer, aging, atherosclerosis, ischemic injury, and inflammation as well as neurodegenerative diseases (Folmer *et al.*, 2014; Nile and Park, 2014; Pal and Verma, 2013). Drying is one of the traditional methods used for the conservation of fruits and vegetables (Doymaz and İsmail, 2011). It is safe and environmentally friendly, as it does not use chemical preservatives. The advantage of food dehydration is that it reduces the moisture content and the water activity value, which prevents the proliferation of microorganisms and the degradation reactions of food (Vega-Gálvez *et al.*, 2009). Traditional sun drying, currently used in southern Spain (Region of Montilla-Moriles) and southern Italy for grape raisining, depends on the environmental conditions and is a process that can take a few days and even weeks or months to be carried out. However, it presents several disadvantages. In recent years, different methods have been developed to control conditions and obtain higher quality products. The chamber-drying methods with hot air at controlled temperature, are reliable and easy to use. They also avoid the disadvantages caused by traditional drying, such as time, losses caused by the attack of insects, climatic changes such as occasional

rains or strong solar radiation, or the deterioration of products by the attack of fungal toxins such as ochratoxin A (Marquez *et al.*, 2012). Authors such as Martin-Gomez *et al.* (Martin-Gomez *et al.*, 2017) and Marquez *et al.* (Marquez *et al.*, 2014) have shown that the partial drying of berries is a good preservative method, as well as, increasing the concentration of phenolic compounds and the antioxidant activity of the obtained fruits.

The aims of this work were to evaluate the improvement in drying performance by piercing the structure of the berry, developing predictive models of the kinetics of chamber-drying with hot air and observing the drying effect on the total phenolic compounds and antioxidant activity of the fruits.

MATERIALS AND METHODS

Materials

Grapes of Tempranillo variety (*Vitis vinifera* L.) were purchased by the Andalusian Institute for Research and Training in Agriculture, Fisheries, Food and Organic Production (IFAPA, Cabra, southern Spain). The grapes were harvested at the commercial optimum maturity stage, then were labeled and stored at -18 °C. For thawing, the grapes were kept at 25 ° C for 24 hours. The grapes were destemmed and divided into two batches. In the first, the destemmed grapes kept the pedicel (whole grapes), in the second batch the pedicel were removed from the destemmed grapes, so they had a perforation in the structure of the berry (punched grapes).

The initial moisture content of the grapes was obtained by the AOAC method (AOAC, 1996), where the grapes were dried at 100 ºC until constant weight. This value was 2.70 Kg of water/Kg of dry matter (Kg water/Kg d.m.).

Drying process

An amount of 3 kg of punched and whole grapes were dried in triplicate in a chamber at constant temperature and an initial relative humidity of 20% (Figure 1). Both types of grapes (whole and punched grapes) were dried at 30, 40 and 50 ºC, the relative humidity increased in the first two hours of drying to 42% and then decreased to 20% to the end of the process. All the drying processes were carried out for 72 hours, performing a periodical weight loss control.

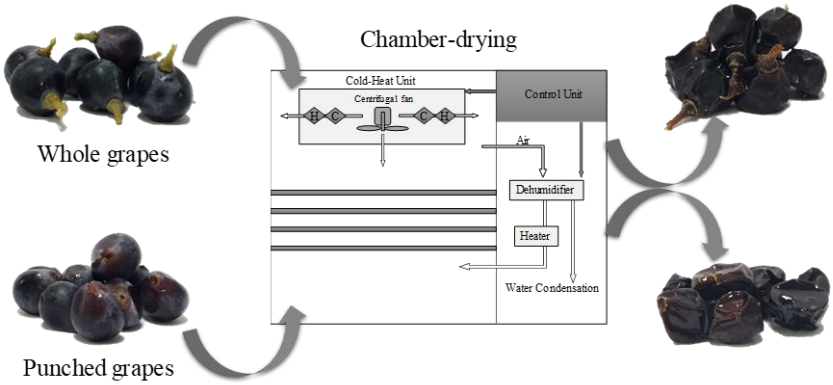

Figure 1. Scheme of the chamber- drying and the changes produced in the grapes.

Mathematical models

The data obtained from the drying processes were adjusted to different mathematical models frequently used to model drying curves. For this, the moisture ratio (MR) was calculated using the following formula:

$$MR=\frac{M_t - M_e}{M_0 - M_e} \quad (1)$$

where Mt, M_0 and Me are the moisture content at any time of drying (kg water/kg d.m.), initial moisture content (kg water/kg d.m.) and equilibrium moisture content (kg water/kg d.m.), respectively. The moisture content in the equilibrium is small relative to the others, it can be assumed that the equation 1 can be simplified as follows (Dissa *et al.*, 2011; Doymaz, 2017; Doymaz *et al.*, 2006; Jahedi Rad *et al.*, 2018):

$$MR=\frac{M_t}{M_0} \quad (2)$$

The data were analyzed with Statgraphics Centurion XVI.I software, where the model constants and coefficients were determined by non-linear regression, based on the Marquardt algorithm. To evaluate the adjustment of the mathematical models with the experimental data, the coefficient of determination (R^2), the reduced chi square (χ^2) and the root mean square error (RMSE) were used, a high value of R^2 and a low value of χ^2 and RMSE establish a better fit. These parameters were calculated with the following equation (Doymaz and İsmail, 2011):

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{\sum_{i=1}^{N}\left(\overline{MR}_{exp} - MR_{exp,i}\right)^2} \quad (3)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{n-z} \quad (4)$$

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2\right]^{\frac{1}{2}} \quad (5)$$

Where $MR_{exp,i}$ and $MR_{pre,i}$ are the experimental and predicted Moisture ratio respectively, N the number of observations and z the constant numbers.

Determination of effective diffusivity

The effective diffusivity was calculated for each drying process, using the analytical solution of Fick's second law for spherical geometry assuming that the shape of the grape is spherical, the shrinkage of the fruit negligible, the migration of humidity is due to diffusion and constant coefficients and temperature (Crank, 1979; Doymaz, 2007; Doymaz and İsmail, 2011; Essalhi *et al.*, 2018; López *et al.*, 2017).

$$MR = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{n^2\,\pi^2 D_{eff}\,t}{R^2}\right) \quad (6)$$

Where D_{eff} is the effective diffusivity (m^2/s), R is the equivalent radius of the grape (m) and t is time (s). When the drying process is long the equation can be simplified.

$$Ln\,(MR) = Ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{R^2}\right)t \quad (7)$$

In order to calculate the effective diffusivity coefficient from the previous equation, it is necessary to represent the natural logarithm of the moisture relation versus time. The slope of the line they form is given by:

$$Slope = \frac{D_{eff}\;\pi^2}{R^2} \quad (8)$$

Calculation of activation energy

The effective diffusivity depends on the temperature and is calculated from the Arrhenius equation (Simal *et al.*, 2005).

$$D_{eff} = D_0 \exp\left(-\frac{E_a}{R\,(T+273.15)}\right) \quad (9)$$

Where D_0 is the pre-exponential factor of the Arrhenius equation (m^2/s), E_a is the activation energy (KJ/mol), R is the universal gas constant (KJ/mol K) and T is the temperature of air (°C).

The activation energy is calculated from the slope of the line of the natural logarithm of the effective diffusivity coefficient versus the inverse of the temperature.

$$\mathrm{Ln}\,(D_{eff}) = \mathrm{Ln}\,(D_0) - \frac{E_a}{R\,(T+273.15)} \quad (10)$$

$$\text{Slope} = -\frac{E_a}{R} \quad (11)$$

Extraction process

0.2 g of fresh grapes or 0.1 g of dried grapes were treated with 3 mL of acidified methanol (0.1% HCl), introducing the mixture in ultrasound for 10 minutes. The supernatant was removed and added 3 mL of acidified methanol again performing the procedure three times. All the extract was centrifuged 10 minutes at 4000 rpm and the supernatant was made up to 10 mL. The extract was filtered with 0.45 µm nylon filter before analysis.

Total phenolic compounds (TPC)

The total phenolic content was estimated in triplicate with the Folin Ciocalteau method with some modifications. 1.250 mL of Folin Ciocalteau reagent diluted 1:5 in distilled water, was added to 50 µL of extract filtered through 0.45 µm. After vigorous agitation and incubation for 1 minute at room temperature (25 ºC), 1 mL of 10% sodium carbonate was added. The mixture was again stirred and allowed to react for 30 minutes in darkness at room temperature. After this time, the absorbance at 760 nm was measured using a UV-vis spectrophotometer (Beckman DU 640). A gallic acid calibration curve was performed using different concentrations of gallic acid standard (1, 0.75,

0.5, 0.25, 0.1, 0.05 and 0.01 mg gallic acid/mL). Total phenolic content was expressed in mg gallic acid/g fruit.

Antioxidant activity

The antioxidant activity was analyzed in triplicate through the DPPH assay according to Katalinic *et al.* (Katalinic *et al.*, 2006) with some modifications. A 45 mg/L solution of DPPH in methanol was prepared on a daily basis and stored in darkness. The analytical procedure was as follows: a 200 µL aliquot of extract filtered through 0.45 µm was placed in a cell and 3 mL of a 45 mg/L solution of DPPH in methanol was then added. A control sample (200 µL of water + 3 mL of DPPH solution) was also prepared in parallel. Following vigorous stirring, the absorbances at 517 nm of the control sample was measured in a Beckman DU 640 spectrophotometer. The sample was measured under identical conditions after 30 min of incubation at room temperature. A Trolox calibration curve was performed using concentrations between 10 and 200 mg Trolox/l. The results were expressed as Trolox equivalents (mg trolox/L) using the following equation to calculate the inhibition percentage:

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \cdot 100 \quad (12)$$

RESULTS AND DISCUSSION

Drying characteristic of Tempranillo Grape

The drying process was represented by the moisture ratio, determined as the ratio of the moisture content at each time to the initial moisture content, versus the drying time (Figure 2). As can be seen the moisture ratio decreased exponentially over time.

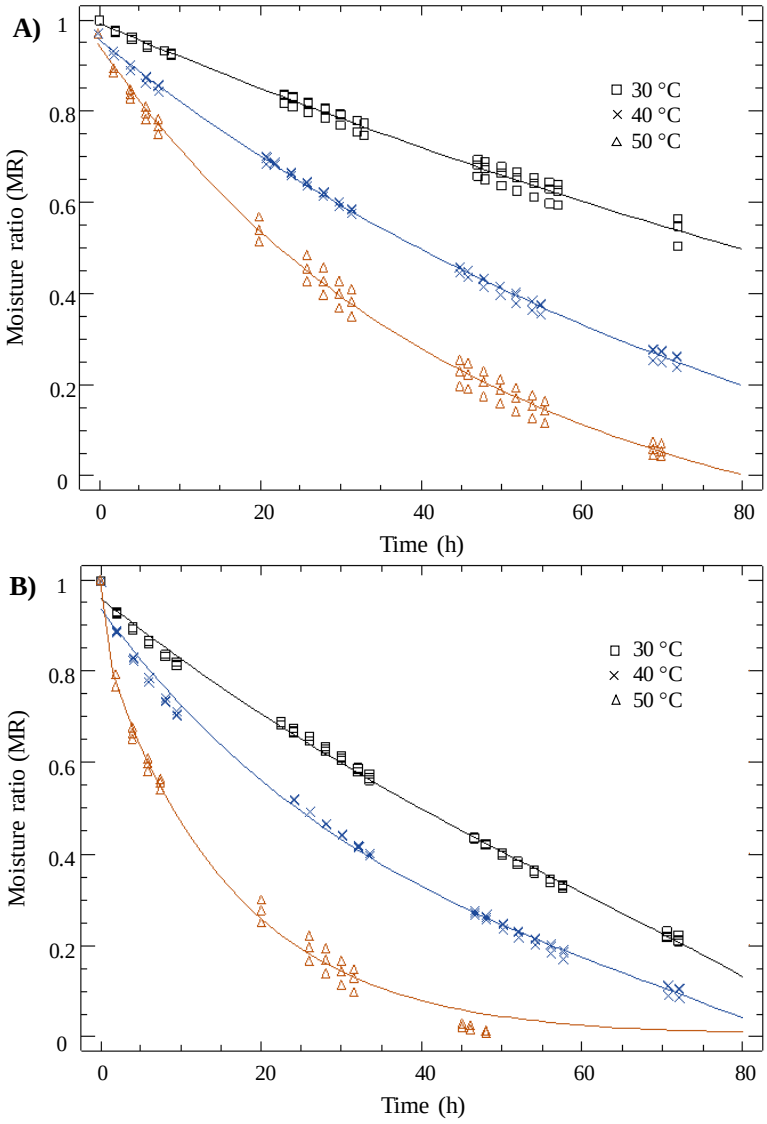

Figure 2. Changes in moisture ratio during the controlled dehydration processes, of whole grapes at 30 °C, 40 °C and 50 °C (A), and punched grapes at 30 °C, 40 °C and 50 °C (B).

Figure 2A shows the temperature effect on the drying time in the whole grapes or grapes with the pedicel. As can be seen in the figure, the higher temperature produced a greater decrease in the humidity ratio. The moisture ratio value was 0.538 at 30 °C at 72 hours of drying, and comparing this value in the other

processes, the process was found to be reduced by 46% and 70% at 40 and 50 °C respectively. This fact implied that the exposure of the berry to a higher temperature favored the process of water elimination. Figure 2B shows the changes in moisture ratio of the punched grapes at the same temperatures. In this case the value of moisture ratio at 30 °C at 72 hours of drying was 0.214. This value at 40 °C was reached in a time 26% lower, and at 50 °C in a time 68% lower. The temperature increased the water evaporation rate of the fruit.

Figure 2 shows the effect of the fruit damage in the drying process kinetics. Comparing the drying processes at 30 °C, the whole grapes needed 72 hours to reach a moisture ratio of 0.538, and the punched grapes reached this value in 36 hours, the process was achieved in half the time. In the processes at 40 °C, the whole grapes reached a moisture ratio of 0.281 at the end of the drying, being this value reached in 45 hours by the punched grapes (37.5% off). Finally, at the temperature of 50 °C, the punched grapes took 47% less time to reach the minimum moisture ratio value obtained by whole grapes. The data obtained indicate that the perforation of the berry favors the water evaporation, minimizing the resistance offered by the wax of the grape skin to the water evaporation (Saravacos *et al.*, 1986). Authors such as Serratosa *et al.* (Serratosa *et al.*, 2008) reduced the drying time by 24%, using a pre-treatment by dipping the fruit in alkaline solutions to reduce the skin's resistance, while the loss of the integrity of the berry can produce even a 50% decrease in the drying time.

Mathematical modeling of drying curves

Table 1. Selected drying models for describing grapes drying processes.

Model name	Model	Reference
Newton	$MR = \exp(-kt)$	(Bruce, 1985)
Henderson and Pabis	$MR = a \exp(-kt)$	(Henderson and Pabis, 1961)
Logarithmic	$MR = a \exp(-kt) + c$	(Yağcıoğlu *et al.*, 1999)
Wang and Singh	$MR = 1 + at + bt^2$	(Wang and Singh, 1978)
Two term	$MR = a \exp(-k_0 t) + b \exp(-k_1 t)$	(Madamba *et al.*, 1996)
Aproximation of diffusion	$MR = a \exp(-kt) + (1-a) \exp(-kbt)$	(Yaldiz *et al.*, 2001)
Midilli et al.	$MR = a \exp(-kt) + bt$	(Midilli *et al.*, 2002)

Table 2. Statistical analysis at different temperatures for whole grape drying.

Model	Temperature (ºC)	χ^2	RMSE	R^2
Newton	30	0.000209	0.014353	0.9887
	40	0.000238	0.015326	0.9952
	50	0.000756	0.027178	0.9914
Henderson and Pabis	30	0.000211	0.014302	0.9887
	40	0.000239	0.015233	0.9953
	50	0.000765	0.027177	0.9914
Logarithmic	30	0.000242	0.015169	0.9873
	40	0.000097	0.009651	0.9981
	50	0.000431	0.020202	0.9952
Wang and Singh	30	0.000205	0.014088	0.9891
	40	0.000113	0.010492	0.9977
	50	0.000759	0.027255	0.9914
Two term	30	0.000196	0.013562	0.9899
	40	0.000079	0.008641	0.9985
	50	0.000439	0.020212	0.9952
Aproximation of diffusion	30	0.000209	0.014134	0.9890
	40	0.000155	0.012194	0.9969
	50	0.000724	0.026205	0.9920
Midilli et al	30	0.000167	0.012935	0.9902
	40	0.000075	0.008477	0.9985
	50	0.000043	0.020183	0.9953

The experimental values of the moisture ratio obtained from the drying processes at different temperatures were adjusted to 7 mathematical models (Bruce, 1985; Henderson and Pabis, 1961; Madamba *et al.*, 1996; Midilli *et al.*, 2002; Wang and Singh, 1978; Yağcıoğlu *et al.*, 1999; Yaldiz *et al.*, 2001) (Table 1). The values of the statistical parameters are shown in Tables 2 and 3. In all cases, the correlation coefficient for the models was above 0.97, indicating a good fit. The R^2, χ^2 and RMSE values were between 0.9762 and 0.9985, 0.000043 and 0.007671, 0.008477 and 0.085120, respectively. The simplicity of the model could be used to select the best fit when the statistical parameters are very closed, however the best fit was stablished for the model with the highest value of R^2 and lowest value of χ^2 and RMSE, which was used

as the optimal criterion to evaluate the quality of the adjustment to the proposed models. Therefore, based on the values of R^2, χ^2 and RMSE, it was shown that the *Midilli et al.* model was the model with a better fit and the one that allowed a better description of the drying characteristics for the whole grape at all the temperatures studied. However, in the case of punched grapes the Two term model was the one that best described the drying characteristics at temperatures of 30 and 40 °C, while the *Aproximation of diffusion* model was the one that presented the best adjustment to the temperature of 50 °C.

Table 3. Statistical analysis at different temperatures for punched grape drying.

Model	Temperature (ºC)	χ^2	RMSE	R^2
Newton	30	0.000803	0,028121	0.9851
	40	0.001013	0.031574	0.9861
	50	0.002352	0.047820	0.9767
Henderson and Pabis	30	0.000778	0.027471	0.9858
	40	0.000579	0.023694	0.9922
	50	0.001422	0.036645	0.9863
Logarithmic	30	0.000296	0.01681	0.9947
	40	0.000415	0.019885	0.9945
	50	0.001462	0.036611	0.9863
Wang and Singh	30	0.000548	0.023059	0.9900
	40	0.001765	0.041349	0.9762
	50	0.007671	0.085120	0.9767
Two term	30	0.000176	0.012864	0.9969
	40	0.000386	0.019016	0.9950
	50	0.000646	0.023969	0.9941
Aproximation of diffusion	30	0.000750	0.026755	0.9865
	40	0.000432	0.020295	0.9943
	50	0.000554	0.022530	0.9948
Midilli et al	30	0.000182	0.013209	0.9967
	40	0.000404	0.019612	0.9946
	50	0.001462	0.036611	0.9863

Determination of the effective diffusivity and activation energy

The values of the effective diffusivity, calculated from the slope of the line obtained by the natural logarithm of the moisture ratio versus time, are shown in Table 4 with a range of values between 8.04 x 10^{-11} and 7.31 x 10^{-11} m^2/s. As can be seen, D_{eff} increased with the increment of air temperature. In addition, the damage of the berries also produced a slight increase of the values of this parameter. The drying at 50 °C of the punched grapes had the highest value of D_{eff} and therefore, the highest water diffusion and evaporation, being the drying of whole grapes at 30 °C, those that presented the lower water diffusion. The D_{eff} values obtained were collected within the general desirable range for the drying of food materials, between 10^{-12} and 10^{-8} m^2/s (Zogzas *et al.*, 1996).

The activation energy is defined as the energy needed to remove 1 mole of water from the material to be dried and is calculated through the slope of the line formed by plotting the natural logarithm of the effective diffusivity versus the inverse of the temperature. The values obtained from this parameter were 56.49 and 54.43 kJ/mol (Table 4) for whole and punched grapes respectively. Since in both cases the studied sample was grape these data resulted similar, even though the corresponding slight difference could be due to the fact that the grapes that had the pedicel needed a greater amount of energy to evaporate the same amount of water from the fruit.

Table 4. Activation energy and effective diffusivity of whole and punched grapes.

Sample	Activation energy (kJ/mol)	Temperature (ºC)	Effective diffusivity (m^2/s)
Whole grape	56.49 ± 6.29	30	8.04 x 10^{-12} ± 6.71 x 10^{-13}
		40	1.66 x 10^{-11} ± 5.83 x 10^{-13}
		50	3.22 x 10^{-11} ± 2.39 x 10^{-12}
Punched grape	54.43 ± 4.00	30	1.90 x 10^{-11} ± 3.27 x 10^{-13}
		40	2.86 x 10^{-11} ± 1.26 x 10^{-12}
		50	7.31 x 10^{-11} ± 6.33 x 10^{-12}

Total phenolic content and antioxidant activity

The total phenolic compounds (TPC) was measured by the Folin-Ciocalteau method in all the drying processes carried out (Figure 3A). As can be seen, the values of TPC increased with the increment of temperature because these processes are influenced by the concentration effect due to the water evaporation. Comparing the treatment of the grapes, the whole grapes presented higher values than punched grapes, except at 50 °C, where the values were similar. The TPC increased 1.86, 2.5 and 2.88 times the initial value for 30, 40 and 50 °C respectively. In the drying of the punched grapes, the increases were 1.48, 1.67 and 2.82 times the initial value for 30, 40 and 50 ºC respectively, lower than in the whole grapes. Considering that the moisture contents were lower in the punched grapes, the concentration effect should be higher in these grapes and the TPC values should be higher, therefore, the composition of damaged grapes (without pedicel) were more affected by the temperature. The increase in total phenolic compounds during drying process of fruit has been reported by authors as Zhao *et al.* (Zhao *et al.*, 2019). They demonstrated that total phenolic compounds increased during the drying of wolfberries at 40, 50 and 60 ºC. However, other authors have reported the decrease of these compounds during the drying of berries as strawberry (Méndez-Lagunas *et al.*, 2017). Some mechanisms can produce the decrease of phenolic compounds for example release of bound phenolic compound, partial degradation of lignin or thermal degradation (Méndez-Lagunas *et al.*, 2017). Authors as Henríquez *et al.* (Henríquez *et al.*, 2014), concluded that the degradation reactions of phenolic compounds increases with the drying temperature, and these compounds could take part in enzymatic and non-enzymatic reactions, the formation of insoluble oxidation compounds, polymer production and thermolabile compound degradation (Obied *et al.*, 2008). The final content of total phenolic compounds is a balance between the concentration effect due to the water evaporation and the mechanisms to reduce their concentration.

Figure 3B shows the antioxidant activity values, measured as the inhibition percentage of the DPPH radical. The values increased during the drying processes, being this increase higher in the whole grapes than in the punched grapes. In whole grapes, the antioxidant activity increased 2.77 times at 30 °C and 3.1 times at 40 and 50 °C. In the drying of punched grapes, the increases were 1.6, 1.9 and 2.7 times at 30, 40 and 50 °C respectively. The antioxidant

activity was related to phenolic compounds. The contents of these compounds reached higher values in the drying of whole grapes, so the antioxidant activity was also higher, although a regression analysis did not show a significate correlation between both values, due to other compounds could be affect the antioxidant activity.

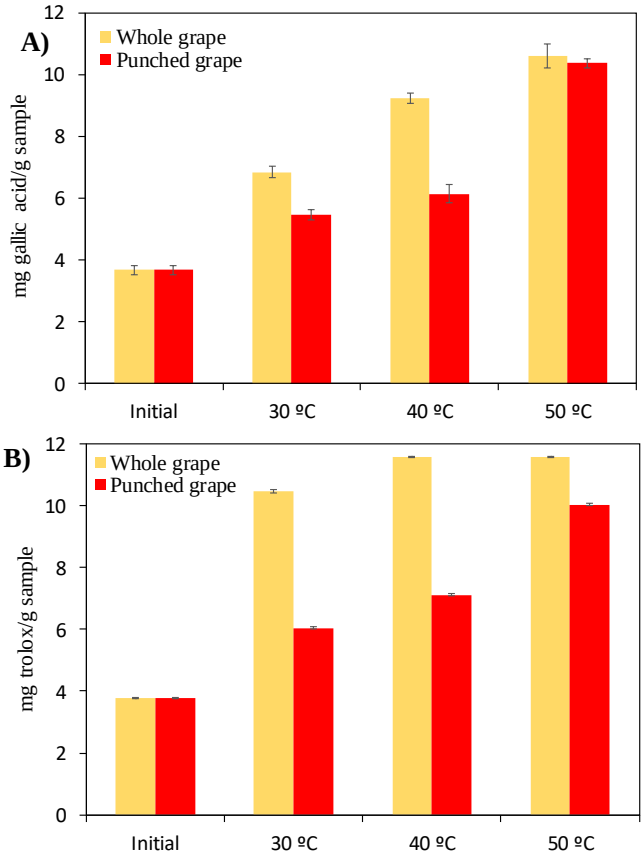

Figure 3. Total phenolic content (A) and Antioxidant activity in drying processes of the whole and punched grapes (B).

CONCLUSIONS

The influence of temperature on the drying kinetics showed a reduction of time with the increase of temperature, being this reduction up to 70% at 50 °C in respect to 30 °C. The grape damage favored the process of

water evaporation, decreasing the drying time by 50% in respect to whole grapes.

The *Midilli et al.* model showed the best fit to describe the drying of whole grapes. However, the punched grapes had a better fit with the Two term model at 30 and 40 °C, and the *Aproximation of diffusion* model at 50 °C.

The effective diffusivity coefficient increased with the temperature and the perforation of the grape and values obtained were in the range from 8.04×10^{-12} to 7.31×10^{-11} m^2/s, and the values of the activation energy showed that less energy was needed to eliminate the water of the punched grapes (54.43 kJ/mol) than of the whole grapes (56.49 kJ/mol).

In relation to the content of total polyphenols and the antioxidant activity, an increase was observed in all the drying processes compared to the initial content, due to the concentration effect by the loss of water. But it was in the drying processes of whole grapes where the phenolic compounds were more stable in respect to the temperature and more content was obtained.

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CAPÍTULO V

RESULTADOS Y DISCUSIÓN

Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (*Vaccinium corymbosum*)

ÍNDICE

LWT – Food Science and Technology

Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (*Vaccinium corymbosum*)

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ABSTRACT

This work studies blueberry convective drying-process at 30, 40 and 50 ºC, with the aim to obtain juices with the highest concentration of phenolics compounds and antioxidant activity. To predict changes in moisture content versus drying time, the results were adjusted to 5 mathematical models to evaluate the best fit, which is the one with the highest value of the coefficient of determination (R2) and lowest values the reduced chi square ($\chi 2$) and the root mean square error (RMSE). The *Page* model was the best model for the drying processes at 30 and 50 ºC, while at 40 ºC, the best model was the *Approximation of diffusion* model. The values of effective moisture diffusivity ranged between 2.58 x 10-11 and 4.58 x 10-11 m^2/s, and the activation energy was 23.13 kJ/mol. The increase in temperature increased the absorbances at 420, 520 and 620 nm of the juice, the total phenolic contents (TPC) values and the anthocyanin concentration. The antioxidant activity, evaluated by the DPPH and ABTS methods, increased with the drying temperature. The best TPC values, antioxidant activity, anthocyanins concentration and color

parameters were obtained from blueberries dried at 50 ºC, being the most effective process in removing water.

KEYWORDS

Blueberry, kinetic drying, phenolic compounds, anthocyanins, antioxidant activity

1. INTRODUCTION

The most cultivated blueberries worldwide belong to the genus *Vaccinium* and species *corymbosum* and are native to North America and regions of Europe (Reque *et al.*, 2014). These blueberries are of high quality and comprise 85% of the world production of the fruit (Garcia *et al.*, 2013; Miyashita *et al.*, 2018). The American continent is the largest producer of blueberries, accumulating three-quarters of the world production (Hidalgo and Almajano, 2017). Blueberries are small berry fruits that are sweet and juicy with an intense blue color and high content of phenolic compounds, such as phenolic acids, tannins, stilbenes, lignans and flavonoids, including anthocyanins, flavonols and flavanols (Guiné *et al.*, 2018; Nile and Park, 2014).

Phenolic compounds are known to have beneficial health properties; anthocyanins, the pigments responsible for the color of the berries, can represent up to 70% of the phenolic compound contents of blueberries (Olas, 2018). They are antioxidant compounds that can react with reactive oxygen species (ROS) to reduce the effects of oxidative stress, which gives them anti-inflammatory, cardioprotective, anticancer, neuroprotective, anti-aging, oculo-protective, and renal-protective properties. They also function against type 2 diabetes and have other dietary properties, reducing the risk of obesity (Das and Mandal, 2018; Fairlie-Jones *et al.*, 2017; Lee *et al.*, 2017; Leong *et al.*, 2018; Pavlidou *et al.*, 2018; Różańska and Regulska-Ilow, 2018; Singh *et al.*, 2018).

Drying is one of the oldest food preservation methods, since reducing the moisture content of food prevents the proliferation of microorganisms (Vega-Gálvez *et al.*, 2009). In this process, the application of energy is necessary to achieve a change of state, sublimation or evaporation inside the food, resulting in water loss (Nemzer *et al.*, 2018). Different types of drying can be applied

according to the method of heat transfer used, such as drying by conduction, convection or radiation (Kudra and Mujumdar, 2009).

From an oenological point of view, drying is a very important process in different parts of the world, such as southern Spain or southern Italy. Since many wines are made from dried grapes, radiation drying has traditionally been the most widely used, where the sun is the carrier of thermal energy; this process is known as "soleo". This type of drying has many disadvantages, such as losses due to inclement weather (rain, hail etc.), variable temperatures at different times of day, and losses due to insect attacks, among others (Marquez *et al.*, 2014, 2012; Serratosa *et al.*, 2008). There are different types of controlled drying, such as convection drying in a chamber, where a constant temperature and humidity value can be adjusted to desired levels, and the disadvantages discussed above are minimized. Authors such as Serratosa *et al.* (Serratosa *et al.*, 2008) showed that in the drying of grapes, in which drying by "soleo" is still used, chamber-drying resulted in products with reduced loss of bioactive compounds, in addition to shortening the exposure time, reducing costs and increasing the quality of the product. The kinetic study of food drying processes is based on the study of the temporal evolution of moisture content in foods. For this, many authors (Henderson and Pabis, 1961; Page, 1949; Wang and Singh, 1978) have defined kinetic models that allow predicting the behavior of foods during drying process.

One of the disadvantages of winemaking of the blueberries is the low sugar content that they present, for this reason after the fermentation would be obtained drinks with an alcoholic content of around 5-6 % (Johnson *et al.*, 2013; Su and Chien, 2007), to avoid this inconvenience the sugar content of the fruit may be concentrated after partial dehydration of the berry (Martin-Gomez *et al.*, 2017). In addition, the fruit drying processes cause the loss of phenolic compounds and antioxidant activity due to the degradation reactions of these compounds that can be favored by the temperature (Lohachoompol *et al.*, 2004; Martin-Gomez *et al.*, 2017). Therefore, the aim of this work was to obtain enriched juices from dried blueberries, with high values of phenolic compounds and antioxidant activity, for this, the influence of temperature on the kinetics of the drying process and on the juice composition was evaluated.

2. MATERIALS AND METHODS

2.1. Chemicals

Folin Ciocalteu reagent, sodium carbonate, acid formic, acetonitrile, potassium peroxodisulfate, ethanol and methanol were obtained from Merck (Madrid, Spain). Anthocyanins were purchased by Extrasynthese (Genay, France). Gallic acid, DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and Trolox were from Sigma-Aldrich Chemical Co. (Madrid, Spain). All solvents used in this investigation were high-performance liquid chromatography (HPLC) grade.

2.2. Materials

Blueberries of the Ventura variety (*Vaccinium corymbosum*) were purchased by a Company from Huelva (southern Spain). The blueberries were harvested at the commercial optimum maturity stage and stored at -18 °C. For thawing, the blueberries were kept at 25 °C for 24 hours.

The initial moisture content of the blueberries was obtained by the AOAC method (Intl, 2016). The blueberries were then dried at 100 °C until a constant weight was recorded. This value was 5.87 kg of water/kg of dry matter (kg water/kg d.m.).

2.3. Drying process

The blueberries were dried with an initial relative humidity of 20% and a constant air temperature of 30, 40 and 50 ºC in a Frisol Climatronic chamber. Each drying process at different temperatures was performed in triplicate. All the drying processes were carried out by performing periodical weight loss measurements (every 2 hours, during daylight hours) until the berries reached 50% of their initial moisture content, that was, when its moisture content was 2.94 kg water/kg d.m. A laboratory balance with accuracy at 0.02 g was used to achieve these measurements.

The fresh blueberries (after thawing) were manually pressed, obtained the juice (J0), and blueberries dried at 30, 40 and 50 ºC were also pressed manually, to obtain their juices, (J30, J40 and J50 respectively). All juices were centrifuged at 2800 g, filtered with Millipore (Billerica, MA) HA filters of 0.45 μm pore size, and analyzed in triplicate.

2.4. Mathematical models

The data obtained from the drying processes were adjusted to different mathematical models frequently used to model drying curves. For this, the moisture ratio (MR) was calculated using the following equation:

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (1)$$

Where Mt, M_0 and Me are the moisture content at a given drying time (kg water/kg dm), the initial moisture content (kg water/kg dm) and the equilibrium moisture content (kg water/kg dm), respectively. As the equilibrium moisture content is small relative to the others, it can be assumed that it is equal to zero (Doymaz *et al.*, 2006), simplifying the equation to:

$$MR = \frac{M_t}{M_0} \quad (2)$$

The data were analyzed with Statgraphics Centurion XVI.I software, where the model constants and coefficients were determined by nonlinear regression, based on the Marquardt algorithm. To evaluate the fit of the mathematical models and the experimental data, the coefficient of determination (R^2), the reduced chi square (χ^2) and the root mean square error (RMSE) were used, a high value of R^2 and low values of χ^2 and RMSE denote a better fit. These parameters were calculated with the following equations (Doymaz and İsmail, 2011):

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{\sum_{i=1}^{N}\left(\overline{MR}_{exp} - MR_{exp,i}\right)^2} \quad (3)$$

$$\chi 2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{n - z} \quad (4)$$

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2\right]^{\frac{1}{2}} \quad (5)$$

Where $MR_{exp,i}$ and $MR_{pre,i}$ are the experimental and predicted moisture ratios, respectively, N is the number of observations and z is a constant.

2.5. Determination of effective diffusivity

The effective diffusivity was calculated for each drying process using the analytical solution of Fick's second law for spherical geometry, assuming that blueberry shape is spherical, the shrinkage of the fruit is negligible, and the migration of humidity is due to diffusion with a constant coefficient and temperature.

$$MR=\frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{n^2\pi^2 D_{eff}\,t}{R^2}\right) \quad (6)$$

Where D_{eff} is the effective diffusivity (m^2/s), R is the equivalent radius of blueberries (m) and t is time (s). When the drying process is long, the equation can be simplified.

$$Ln\,(MR) = Ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{R^2}\right)t \quad (7)$$

To calculate the effective diffusivity coefficient from the previous equation, it is necessary to represent the natural logarithm of the moisture relation versus time. The slope of the line they form is given by:

$$Slope = \frac{D_{eff}\,\pi^2}{R^2} \quad (8)$$

2.6. Determination of activation energy

The effective diffusivity depends on the temperature and is calculated from the Arrhenius equation (Simal *et al.*, 2005):

$$D_{eff} = D_0 \exp\left(-\frac{E_a}{R\,(T+273.15)}\right) \quad (9)$$

Where D_0 is the pre-exponential factor of the Arrhenius equation (m^2/s), E_a is the activation energy (kJ/mol), R is the universal gas constant (kJ/mol K) and T is the air temperature (°C).

The activation energy is calculated from the slope of the line of the natural logarithm of the effective diffusivity coefficient versus the inverse of the temperature.

$$\text{Ln}\,(D_{eff}) = \text{Ln}\,(D_0) - \frac{E_a}{R\,(T+273.15)} \quad (10)$$

$$\text{Slope} = -\frac{E_a}{R} \quad (11)$$

2.7. Spectrophotometric determinations

The optical density of the juices from undried (J0) and dried blueberries at 30 ºC (J30), 40 ºC (J40) and 50 ºC (J50) were measured on a UV-vis spectrophotometer (Beckman DU 640) using quartz cells with a 1 mm light path, which were corrected for a path length of 1 cm. Absorbance was read at 420, 520 and 620 nm, which represented the contribution of brown, red and blue colors respectively (Martin-Gomez *et al.*, 2017). The hue indicates the relation between the absorbance measured at 420 and 520 nm.

2.8. Total phenolic contents (TPC)

The total phenolic compound values of the juices were estimated using the Folin-Ciocalteu method (Varo *et al.*, 2018). The absorbance at 760 nm was measured using a UV-vis spectrophotometer (Beckman DU 640). A gallic acid calibration curve was performed using different concentrations of gallic acid standard (1, 0.75, 0.5, 0.25, 0.1, 0.05 and 0.01 g gallic acid/L). The total phenolic content was expressed in g gallic acid equivalents/L (g GAE/L).

2.9. Antioxidant activity

2.9.1. DPPH method

The antioxidant activity was analyzed using the DPPH assay according to Katalinic *et al.* (Katalinic, Milos, Kulisic, & Jukic, 2006) with some modifications. A 45 mg/L solution of DPPH in methanol was prepared daily and stored in darkness. The analytical procedure was as follows: a 200 µL aliquot of sample diluted at 1:10 was placed in a cell, and 3 mL of a 45 mg/L solution of DPPH radical in methanol was then added. A control sample (200 µL of water + 3 mL of DPPH radical solution) was also prepared in parallel. Following vigorous stirring, the absorbances of the control sample at 517 nm were measured in a Beckman DU 640 spectrophotometers. The samples were measured under identical conditions after 30 minutes of incubation at room temperature. The antiradical effect was calculated using the equation (12). A

Trolox calibration curve was prepared and the results were expressed as Trolox equivalents (g Trolox/L).

$$\text{Antiradical effect } = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \cdot 100 \qquad (12)$$

2.9.2. ABTS method

Total antioxidant activity was measured by discoloration of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation according to the method described by Re *et al.* (Re *et al.*, 1999). Where ABTS·+ was produced by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate, this mixture was kept in the dark at room temperature for 12–16 hours before use. The resulting ABTS·+ solution was diluted in a 20 mM phosphate buffer at pH 7.4 until the absorbance at 734 nm was 0.700 ± 0.020, as measured in the Beckman DU 640 spectrophotometers. For the analysis of the antioxidant activity, 900 µL of the ABTS·+ solution dilution was mixed with 100 µL of the sample diluted to 1:100. After 6 min, the absorbance at 734 nm was measured next to a blank with distilled water. The antiradical effect was calculated using the equation (13). A Trolox calibration curve was prepared and the results were expressed as Trolox equivalents (g Trolox/L).

$$\text{Antiradical effect } = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \cdot 100 \qquad (13)$$

2.10. Identification and quantification of anthocyanins by HPLC-DAD

A 50 µL sample of the filtered juice was injected into an HPLC-DAD instrument (Beckman Coulter System Gold, 168 Detector), and the analyses were carried out on a LiChrospher 100 RP-18 column (250 mm×4.6 mm, 5 μm), using 10% aqueous formic acid in HPLC-grade water (solvent A) and 10% formic acid, 45% acetonitrile and 45% HPLC-grade water (solvent B), as a mobile phase, at a flow rate of 1 mL/min. The absorbance at 520 nm was used to quantify anthocyanin, according to the method proposed by Marquez *et al.* (Marquez *et al.*, 2012). The identification was carried out by comparing the retention times of the samples with those of the standards and recording their UV-Vis spectra. For the quantitative analysis of anthocyanins, a calibration curve was obtained by injection of different concentration of

malvidin 3-O-galactoside. All the anthocyanins were quantified as malvidin 3-O-galactoside, which is the main anthocyanin in the studied blueberry.

2.11. Statistical analysis

The results for all samples were subjected to analysis of variance at the 99.0% confidence level, in addition homogeneous groups were calculated in order to stablish significates differences between means. A simple linear correlation has been made between antioxidant activity values and total phenolic content TPC. The software used was the Statgraphics Centurion XVI.

3. RESULTS AND DISCUSSION

3.1. Drying characteristic of blueberry Ventura

The drying processes can be followed through the drying curves, where the change in the moisture relation (MR) versus time is represented. Figure 1 shows the drying curves of the blueberries at different temperatures. An increase in temperature produced a greater rate in the process of removing water from the berries. The drying process was stopped when the moisture content was reduced to 50% of the initial value, a humidity ratio of 0.5. At 50 ºC, this value was reached in 14 hours, this time increased to 24 hours at 40 ºC and to 32 hours at 30 ºC, so the drying process performed at 40 ºC reduced the time by 25% compared to drying at 30 ºC, and drying at 50 °C reduced the time 56% compared to drying at 30 °C and 42% compared to drying at 40 °C.

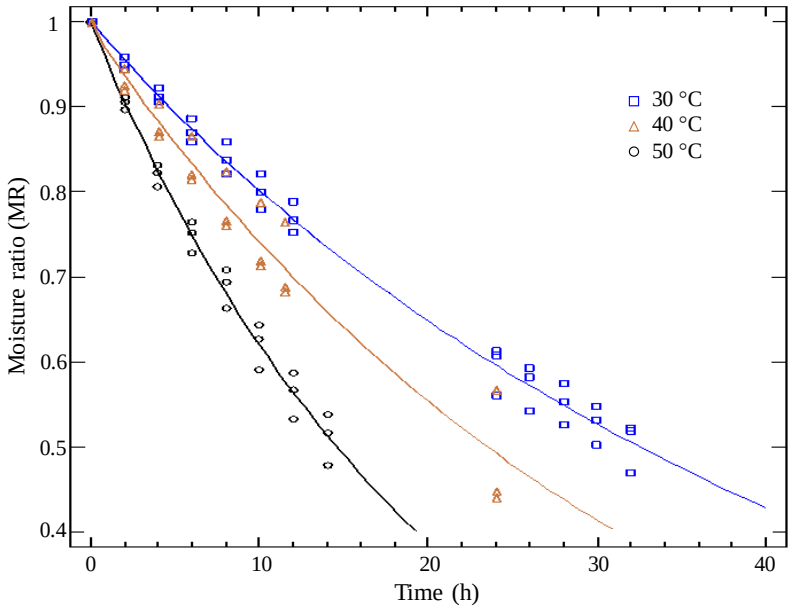

Figure 1. Drying curves of blueberries dried at 30, 40 and 50 ºC.

3.2. Mathematical modeling

The data obtained from the blueberry drying processes at different temperatures were adjusted to 5 mathematical models, as shown in Table 1 (Henderson and Pabis, 1961; Madamba *et al.*, 1996; Page, 1949; Wang and Singh, 1978; Yaldiz *et al.*, 2001).

Table 1. Selected drying models for describing blueberry drying data.

Model name	Model	Reference
Approximation of diffusion	$MR = a \exp(-kt) + (1-a) \exp(-kbt)$	(Yaldiz *et al.*, 2001)
Henderson and Pabis	$MR = a \exp(-kt)$	(Henderson and Pabis, 1961)
Page	$MR = \exp(-kt^n)$	(Page, 1949)
Two term	$MR = a \exp(-k_0 t) + b \exp(k_1 t)$	(Madamba *et al.*, 1996)
Wang and singh	$MR = 1 + at + bt^2$	(Wang and Singh, 1978)

The optimal criteria to evaluate the best fit and the quality of the adjustment to the proposed models was the highest value of R^2 and lowest values of χ^2 and

RMSE. The data obtained for these parameters are shown in Table 2. The R^2 values were between 0.9879 and 0.9908, the χ^2 values were between 0.00030 and 0.00036 and the RMSE values were between 0.01672 and 0.01758, indicating that a good fit was obtained for all the models proposed in Table 1. More specifically, the *Page* model presented the best fit for the drying processes carried out at 30 and 50 ºC, indicating that with this model, the changes in moisture content of the blueberries could be predicted with the drying time. However, the drying performed at 40 °C presented a worse fit to the proposed models, with data ranging between 0.9563 and 0.9573 for R^2, between 0.00112 and 0.00131 for χ^2 and between 0.03124 and 0.03157 for RMSE. In this case, the *Approximation of diffusion* model best described the characteristics of blueberry drying.

Table 2. Statistical analyses for different temperatures for drying processes of blueberries.

Model	Temperature (°C)	χ^2	RMSE	R^2
Approximation of diffusion	30	0.00031	0.01686	0.9907
	40	0.00112	0.03124	0.9573
	50	0.00034	0.01737	0.9882
Henderson and Pabis	30	0.00030	0.01686	0.9906
	40	0.00131	0.03130	0.9571
	50	0.00033	0.01737	0.9882
Page	30	0.00030	0.01672	0.9908
	40	0.00107	0.03136	0.9569
	50	0.00033	0.01736	0.9882
Two term	30	0.00032	0.01675	0.9907
	40	0.00118	0.03130	0.9571
	50	0.00036	0.01737	0.9882
Wang and Singh	30	0.00030	0.01696	0.9905
	40	0.00109	0.03157	0.9563
	50	0.00034	0.01758	0.9879

3.3. Determination of effective diffusivity and activation energy

The effective diffusivity was calculated from the slope of the line obtained by the natural logarithm of the moisture ratio versus the drying time using

equation 8. As seen in Table 3, an increased temperatures produced increased diffusivity values from 2.58 x 10^{-11} at 30 ºC to 4.58 x 10^{-11} m^2/s obtained at 50 ºC, the same order of magnitude that some authors define for spherical products (Kaya *et al.*, 2010). These data showed that an increased temperature produced greater water diffusions within the fruit and a greater evaporation.

The activation energy was calculated from the slope of the line that formed the natural logarithm of the effective diffusivity against the inverse of the drying temperature (equation 11). This parameter is defined as the energy needed to displace 1 mole of water from the sample to be dried. The blueberry variety studied presented an activation energy value of 23.13 kJ/mol, and other authors determined activation energy values between 36.2-54.5 kJ/mol in blueberry varieties (Vega-Gálvez *et al.*, 2009), 57.85 kJ/mol (López *et al.*, 2010) and 61.2 kJ/mol (Shi *et al.*, 2008).

Table 3. Activation energy and effective diffusivity of drying processes of blueberries.

Activation energy (kJ/mol)	**Temperature (ºC)**	**Effective moisture diffusivity (m^2/s)**
23.13 ± 5.40	30	2.58 x 10^{-11} ± 2.04 x 10^{-12}
	40	2.89 x 10^{-11} ± 5.59 x 10^{-12}
	50	4.58 x 10^{-11} ± 4.07 x 10^{-12}

3.4. Spectrophotometric determinations

Color is an important parameter for measuring quality in fruits, and in the processed products derived from them, for example, in fruit juices, it is the first attribute that is perceived and can cause the acceptance or rejection of the food. Figure 2 shows the absorbance values at 420, 520 and 620 nm, which are respectively related to the brown, red and blue-violet compounds of the obtained juices. All of the drying processes were maintained until the initial humidity was reduced by half; therefore, the concentration of the compounds inside the berries due to the evaporation of water should be the same in all cases. All of the juices from dried berries presented an increased absorbance values related to the J0 juice, with the juice from the blueberries dried at 50 ºC presenting the highest absorbance value, this is because at higher drying temperature greater damage occurs in the blueberries skin and the compounds are released and diffused to the blueberries pulp. However, the hue values, which expresses the relation between the absorbance values at 420 and 520 nm

(A420 / A520), did not vary with temperature, obtaining a value of approximately 0.54 ± 0.02 in all cases, indicating that the juices obtained from both the undried and the dried fruits had a greater contribution of red-colored compounds compared to brown-colored compounds.

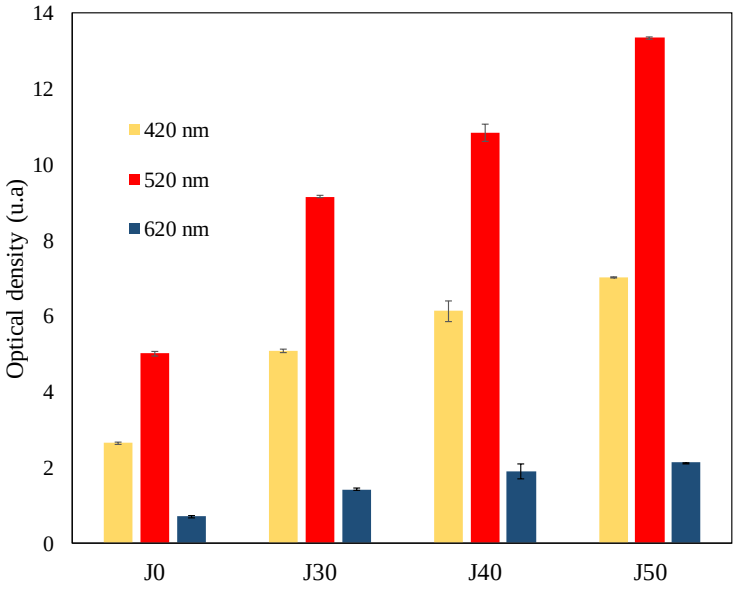

Figure 2. Changes in the absorbances of blueberry juice after the drying process at 420, 520 and 620 nm.

3.5. Total phenolic contents

Phenolic compounds are responsible for several of the health properties of the blueberries; they are found mostly in the skin of the fruits but also in the pulp (Hidalgo and Almajano, 2017). Table 4 shows the TPC values of the juices from undried and dried fruits at different temperatures. The drying process increased the content of phenolic compounds, which was higher at the highest temperature. After the statistical analysis it was found that significant differences existed between all the values. The final moisture content of the dried blueberries at each temperature was approximately the same, so that the concentration factor for the evaporation of water should be the same in all cases, as already discussed above. However, it can be verified that the drying performed at 50 ºC was the temperature group with the highest content,

obtaining 3.35 g GAE/L a value 3 times higher than the value of the juice from undried blueberries. On the other hand, drying at 30 and 40 ºC increased the content of phenolic compounds 1.38 and 1.57 times, respectively, compared to the J0 value. This increase in phenolic compounds may be due to the fact that a higher temperature causes a greater diffusion of the compounds present in the skin into the blueberry pulp due to the cellular damage in the skin from the high temperature (Marquez *et al.*, 2014, 2013). Other authors have demonstrated that the drying process at 45, 65 and 85 ºC reduced the concentration of phenolic compounds in Bunchosia fruits, with greater decreases associated with the increased temperatures (Blank *et al.*, 2018). In strawberries, a decrease in phenolic compounds of 60 and 78% has been observed during convective drying processes at 50 and 60 ºC, respectively (Méndez-Lagunas *et al.*, 2017). During a drying process, several mechanisms can occur that affect the concentration of phenolic compounds, such as the release of bound phenolic compounds and the partial degradation of lignin, leading to the release of phenolic derivatives and thermal degradation (Maillard and Berset, 1995). These reactions, which decrease the concentration of phenolic compounds, together with the concentration process due to the evaporation of water, result in a balance between the mechanisms that increase and those that decrease the concentration of the phenolic compounds.

Table 4. Effect of temperature on total phenolic contents (TPC) and antioxidant activity of juices from undried and dried blueberries.

Sample	TPC (g GAE/L)	Antioxidant activity (g Trolox/L)	
		DPPH assay	ABTS assay
J0	1.12±0.02^{d}	0.63±0.03^{d}	1.08±0.02^{d}
J30	1.54±0.05^{c}	0.79±0.01^{c}	3.76±0.04^{c}
J40	1.76±0.01^{b}	0.90±0.01^{b}	4.63±0.04^{b}
J50	3.35±0.05^{a}	1.04±0.02^{a}	5.13±0.05^{a}

Values in the same column with different superscript letters are significantly different, $p \leq 0.01$.

3.6. Anthocyanin concentrations

Anthocyanins are natural pigments present in the blueberry skins (Olas, 2018; Stevenson and Scalzo, 2012). The anthocyanin profile was determined in the juices from undried and dried blueberries (Figure 3). In Table 5, it can be observed that fourteen compounds were identified in the juice from dried blueberries: the galactoside derivatives of malvidin, peonidin, petunidin, cyanidin and delphinidin; glucoside derivatives of malvidin, peonidin and cyanidin; arabinoside derivatives of malvidin, petunidin, cyanidin and delphinidin; and 2 aglicones (malvidin and cyanidin). Thirteen of the monomers mentioned were found in the undried blueberry juice as well. In all cases, the galactoside, glucoside and arabinoside derivatives of malvidin were the major compounds, malvidin-3-galactoside represented 42% of the total anthocyanins in the J0 juice and approximately 26% in the three dried blueberry juices. Malvidin 3-glucoside represented 22.9% in the fresh juice and approximately 18% in J30, J40 and J50 juices, while the arabinoside derivative represented 16.8% in the J0 juice and approximately 14% in the other three juices. Aglycones presented concentrations below 1 mg/L, and their content was very small in all of the juices tested.

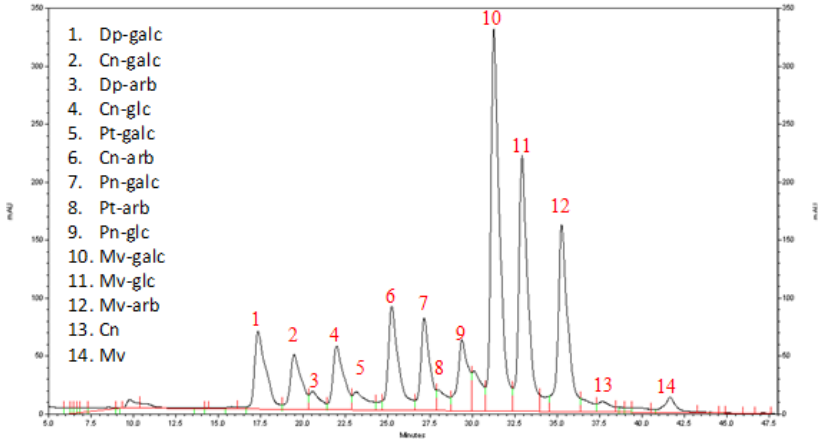

Figure 3. Anthocyanins profile of the juice obtained from blueberry dried at 50 ºC.

Table 5. Anthocyanin concentrations (mg/L) and homogeneous groups of juices from undried and dried blueberries.

Anthocyanins	J0	J30	J40	J50
Mv-galc	11.5 ± 0.09 ^{d}	13.9 ± 0.02 ^{c}	15.3 ± 0.02 ^{b}	19.0 ± 0.03 ^{a}
Pn-galc	0.54 ± 0.01 ^{d}	3.24 ± 0.03 ^{c}	3.97 ± 0.00 ^{b}	4.59 ± 0.01 ^{a}
Pt-galc	0.34 ± 0.01 ^{d}	0.89 ± 0.03 ^{c}	1.01 ± 0.00 ^{b}	1.27 ± 0.02 ^{a}
Cn-galc	n.d.	2.40 ± 0.02 ^{c}	2.99 ± 0.01 ^{b}	3.34 ± 0.04 ^{a}
Dp-galc	0.14 ± 0.01 ^{d}	2.91 ± 0.02 ^{c}	3.29 ± 0.02 ^{b}	4.77 ± 0.02 ^{a}
Total galactosides	12.6 ± 0.13 ^{d}	23.3 ± 0.12 ^{c}	26.6 ± 0.01 ^{b}	33.0 ± 0.07 ^{a}
Mv-glc	6.25 ± 0.06 ^{d}	9.75 ± 0.00 ^{c}	11.1 ± 0.03 ^{b}	12.8 ± 0.01 ^{a}
Pn-glc	0.63 ± 0.01 ^{d}	2.71 ± 0.04 ^{c}	3.21 ± 0.02 ^{b}	4.04 ± 0.03 ^{a}
Cn-glc	0.26 ± 0.02 ^{d}	2.36 ± 0.00 ^{c}	2.89 ± 0.02 ^{b}	3.78 ± 0.01 ^{a}
Total glucosides	7.13 ± 0.09 ^{d}	14.8 ± 0.04 ^{c}	17.2 ± 0.03 ^{b}	20.7 ± 0.03 ^{a}
Mv-arb	4.60 ± 0.00 ^{d}	7.34 ± 0.02 ^{c}	8.66 ± 0.03 ^{b}	10.1± 0.02 ^{a}
Pt-arb	0.53 ± 0.01 ^{c}	0.71 ± 0.02 ^{b}	0.75 ± 0.01 ^{b}	0.96 ± 0.04 ^{a}
Cn-arb	1.40 ± 0.04 ^{d}	4.07 ± 0.02 ^{c}	4.44 ± 0.01 ^{b}	6.00 ± 0.00 ^{a}
Dp-arb	0.67 ± 0.01 ^{b}	0.49 ± 0.01 ^{d}	0.57 ± 0.00 ^{c}	0.92 ± 0.00 ^{a}
Total arabinosides	7.20 ± 0.10 ^{d}	12.7 ± 0.07 ^{c}	14.4 ± 0.00 ^{b}	18.0 ± 0.06 ^{a}
Cn	0.09 ± 0.01 ^{c}	0.50 ±0 .04 ^{b}	0.51 ± 0.02 ^{b}	0.62 ± 0.02 ^{a}
Mv	0.37 ± 0.01 ^{b}	0.69 ± 0.00 ^{c}	0.83 ± 0.01 ^{b}	0.92 ± 0.01 ^{a}
Total aglycones	0.46 ± 0.00 ^{d}	1.19 ± 0.05 ^{c}	1.34 ± 0.03 ^{b}	1.54 ± 0.03 ^{a}
Total	27.4± 0.32 ^{d}	51.9 ± 0.27 ^{c}	59.5 ± 0.07 ^{b}	73.1 ± 0.08 ^{a}

Mv, malvidin; Pn, peonidin; Pt, petunidin; Cn, cyanidin; Dp, delphinidin; galc, galactoside; glc, glucoside; arb, arabinoside; n.d., not detected. Values in the same row with different superscript letters are significantly different, p = 0.01.

The drying process caused an increase in the concentration of anthocyanins, with the magnitude of the increase in anthocyanin concentration increasing with the temperature. The juice obtained from fruits dried at 50 ºC presented the highest concentration of total anthocyanins (73.13 mg/L), 2.67 times greater than the concentration in the undried fruit juice. The increases in J30 and J40 were 1.90 and 2.18 times that of the J0 juice, respectively. Other authors have demonstrated that the drying processes at high temperatures causes a decrease in the anthocyanin contents of strawberries and blueberries (Méndez-Lagunas *et al.*, 2017). As discussed above and considering the same effect of concentration, the temperature favors the diffusion of anthocyanins from the skin to the pulp. Furthermore, the drying process occurs at a moderate temperature, so thermal degradation reactions of the anthocyanins are not highly favored. On the other hand, anthocyanins are also degraded enzymatically in the presence of polyphenol oxidase (PPO), and these enzymes that produce oxidation of phenolic compounds can decrease their activity with increasing temperatures. Exists a balance between the mechanisms that increase and those that decrease the anthocyanin concentration, as previously mentioned.

3.7. Antioxidant activity

The antioxidant activity of the juices was determined by 2 methods, the DPPH and ABTS assays. As shown in Table 4, the increase in drying temperature produced an increase in the antioxidant activity values of the juices, significant differences existed between all values. The juice from fruits dried at 50 °C showed the highest antioxidant activity, 1.04 g Trolox/L and 5.13 g Trolox/L for the DPPH and ABTS assays, respectively. The results obtained by the different assays are not directly comparable due to the difference in the mechanisms of radical capture that occur in each method (Chaves *et al.*, 2018). To evaluate the influence of phenolic compounds on antioxidant activity, a correlation study between the total phenolic compound values and the antioxidant activity was carried out, and the results are shown in Figure 4. The coefficient of determination (R^2) for the DPPH method was 0.8073, which indicates that there was a strong relationship between the antioxidant activity measured by this method and the content of phenolic compounds. The coefficient of determination for the ABTS method was 0.5671, which indicates that there may be other compounds that were not determined by the

Folin-Ciocalteu method that interact with the radical $ABTS^{\cdot+}$ and have antioxidant capacities.

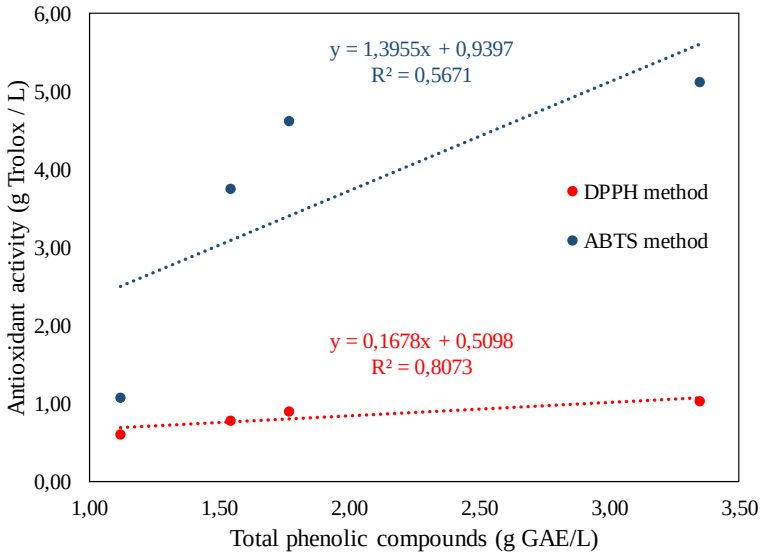

Figure 4. Correlations between total phenolic contents and antioxidant activity values.

4. CONCLUSION

The increase of the drying temperature caused a decrease in the processing time up to 56% in the case of 50 ºC with respect to drying at 30 ºC. The processes of drying blueberries in a controlled temperature chamber is well suited to several kinetic models, with the *Page* model best describing the behavior of the blueberry moisture content in the drying at 30 and 50 ºC, while the *Approximation of diffusion* model presented the best fit for drying at 40 ºC. The effective diffusivity coefficient increased with increasing temperatures, which indicates that the temperature facilitates the diffusion of water through the fruit.

The final moisture ratio of the drying processes was 0.5, so the concentration effect of the fruit was the same in all cases. Despite this, the color, the total phenolic compounds, the anthocyanin content and the antioxidant activity increased as the drying temperature increased. Drying at 50 ºC yielded the

highest values for the absorbances at 420, 520, and 620 nm, the highest content of phenolic compounds (3.35 g GAE/L), the highest concentration of total anthocyanins (73.13 mg/L) and the highest antioxidant activity with 1.04 g Trolox/L and 5.13 g Trolox/L for the DPPH and ABTS methods, respectively. There is a balance between the mechanisms that increase (such as the concentration effect by water evaporation) and those that decrease (such us the degradation reaction of phenolic compounds) the concentration of the compounds and the antioxidant activity values.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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CAPÍTULO VI

RESULTADOS Y DISCUSIÓN

Phenolic compounds, antioxidant activity and color in the fermentation of blueberry and grape juice with different yeasts.

Phenolic compounds, antioxidant activity and color in the fermentation of blueberry and grape juice with different yeasts.

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RESUMEN

En este trabajo, se ha estudiado la influencia de tres cepas de levaduras comerciales, QA23, Elegance MP061 y M05 Mead; y una cepa aislada de mostos parcialmente fermentados de uva Pedro Ximenez, X5, en la fermentación de zumos de mezcla de uvas y arándanos en una proporción 1:1. Las fermentaciones se llevaron a cabo con 100 mL de una mezcla de zumo uva y arándano en un baño termostatizado a 25 ºC y con una carga celular de $5x10^6$ células/mL. El objetivo era evaluar el contenido de compuestos fenólicos, la actividad antioxidante y el color en los vinos elaborados a partir de las diferentes levaduras. Siendo el vino elaborado con la levadura M05 Mead el que obtuvo la mayor concentración de antocianos (55.76 mg/L) y flavonoles (22.60 mg/L) y una menor pérdida de compuestos fenólicos, color y actividad antioxidante.

CAPÍTULO VII

RESULTADOS Y DISCUSION

Fermentation of blueberry and grape dried mix juices. Color, phenolics compounds and antioxidant activity.

Fermentation of blueberry and grape dried mix juices. Color, phenolics compounds and antioxidant activity.

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RESUMEN

El objetivo de este trabajo fue estudiar la fermentación de mostos procedentes de la mezcla de arándanos y uvas, pero tras el proceso de deshidratación de una de las frutas de la mezcla para enriquecer los mostos en azúcar, compuestos fenólicos y actividad antioxidante. La deshidratación de los frutos se llevó a cabo en un cámara de secado con aire a 50 ºC, hasta que las frutas perdieron el 50 % de la humedad inicial. Las fermentaciones se realizaron con 100 mL de mosto (M1, mosto de uva y arándano deshidratado; M2, mosto de arándano y uva deshidratada), y en presencia de 50 g de residuos sólidos del prensado, en un baño termostatizado a 25 ºC y con una carga celular de $5x10^6$ células/mL, de las levaduras X5 (CECT131015) y M05 Mead. Los mostos de partida (M1 y M2) presentaron diferentes contenidos en azúcar por lo tanto los tiempos de fermentación y maceración fueron diferentes. En cambio, los vinos obtenidos presentaron valores de compuestos fenólicos y actividad antioxidante muy similares. Sin embargo, fue la levadura M05 Mead la que fermentó los vinos con el mayor contenido en actividad antioxidante y compuestos fenólicos. Desde el punto de vista sensorial, el parámetro mejor evaluado fue el color, seguido del aroma y, por último, el sabor, siendo el vino W2M el mejor evaluado por los catadores expertos y consumidores habituales de vino, por lo tanto, el de mayor calidad organoléptica.

CAPÍTULO VIII

CONCLUSIONES / CONCLUSIONS

CONCLUSIONES

En el primer trabajo descrito en el capítulo III de la Memoria de Tesis Doctoral, titulado **"*Bioactive compounds of chamber-dried blueberries at controlled temperature and wines obtained from them*"**, se llegaron a las siguientes conclusiones:

1. El proceso de secado aumentó las absorbancias a 420, 520 y 620 nm, así como el índice de pardeamiento, la contribución de compuestos de color rojo, medida con la tonalidad, y el contenido en antocianos y taninos debido a la mayor difusión de los compuestos de la piel hacia la pulpa. En cambio, disminuyó el contenido en flavonoles, favan-3-oles y vitamina C.
2. El proceso fermentativo produjo una disminución de compuestos fenólicos (antocianos, flavonoles, flavanoles y taninos) y actividad antioxidante, mientras que la vitamina C se mantuvo constante.
3. De los resultados obtenidos del análisis sensorial, los vinos elaborados que presentaron azúcar residual tras la fermentación son aquellos que recibieron las mejores valoraciones en sabor y aroma. Estando el parámetro del color bien valorado en todos los vinos.
4. El vino fermentado hasta un 14 % v/v de alcohol fue el vino mejor valorado desde el punto de vista sensorial, y aquél que presentó los mayores contenidos en compuestos fenólicos y actividad antioxidante.

En el segundo trabajo descrito en el capítulo IV de la Memoria de Tesis Doctoral, titulado ***"The influence of berry perforation on grape drying kinetic and total phenolic compounds"***, se llegaron a las conclusiones:

5. El aumento de la temperatura de secado redujo el tiempo del proceso hasta un 70 % a 50 ºC con respecto del secado a 30 ºC.
6. El daño provocado en la baya al quitarle el pedicelo favoreció la difusión del agua y permite disminuir el tiempo de secado hasta en un 50 % con respecto de las uvas que se deshidrataron enteras.
7. El modelo de *Midilli et al.* ($MR = a \exp(-kt) + bt$) mostró el mejor ajuste para describir las características de secado de las uvas enteras. No obstante, para las uvas perforadas el modelo de *Two term* ($MR = a \exp(-k_0 t) + b \exp(-k_1 t)$) fue el que presentó el mejor ajuste para las temperaturas de 30 y 40 ºC y el modelo de *Aproximation of diffusion* ($MR = a \exp(-kt) + (1-a) \exp(-kbt)$) para la temperatura de 50 ºC.

8. El coeficiente de difusividad efectiva manifestó la difusión del agua, y se demostró que este era mayor conforme se aumentaba la temperatura de secado, así como cuando la uva estaba perforada. Además, se comprobó que la energía necesaria para eliminar un mol de agua del fruto (energía de activación) era mayor para las uvas enteras (56.49 kJ/mol) que para las uvas perforadas (54.43 kJ/mol).
9. El contenido en compuestos fenólicos y, por ende, la actividad antioxidante de las uvas aumentaba tras el proceso de secado con respecto a las uvas de partida, debido al efecto de preconcentración de los compuestos por la pérdida de agua y a la mayor difusión de estos desde los hollejos a la pulpa. Siendo las uvas enteras las que presentaron los mayores valores y, por lo tanto, una mayor estabilidad térmica de los compuestos fenólicos durante el secado.

En el tercer trabajo descrito en el capítulo V de la Memoria de Tesis Doctoral, titulado ***"Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (Vaccinium corymbosum)"***, se llegaron a las conclusiones que aparecen a continuación:

10. Se redujo el tiempo de secado con el aumento de temperatura, hasta un 56% en el caso del secado a 50 ºC con respecto al de 30 ºC.
11. El modelo de *Page* ($MR = \exp(-kt^n)$) fue el mejor prediciendo los cambios de humedad en los secados a las temperaturas de 30 y 50 ºC, mientras que para el secado a 40 ºC el mejor ajuste lo presentó el modelo de *Aproximation of diffusion* ($MR = a \exp(-kt) + (1-a) \exp(-kbt)$).
12. El coeficiente de difusividad efectiva aumentó con la temperatura, lo que indicó que la temperatura facilitó la difusión del agua desde la fruta.
13. A pesar de que todos los arándanos fueron secados hasta que perdieron el 50 % de la humedad inicial, y, por lo tanto, tuvieran el mismo factor de preconcentración por la evaporación del agua, se observaron diferencias entre los secados. Siendo el secado a 50 ºC el que presentó las mayores absorbancias a 420, 520 y 620 nm, el mayor contenido en compuestos fenólicos y la más alta actividad antioxidante medida tanto por el ensayo ABTS como DPPH.

En el cuarto trabajo descrito en el capítulo VI de la Memoria de Tesis Doctoral, titulado ***"Phenolic compounds, antioxidant activity and color in the fermentation of blueberry and grape juice with different yeasts"***, se llegaron a las siguientes conclusiones:

14. El uso de inóculos de levadura permitió reducir el tiempo de fermentación, saltando la fase de latencia del crecimiento de las levaduras y evitando la aparición de posibles reacciones secundarias.
15. El proceso de fermentación sin maceración obtuvo vinos con una pérdida de intensidad colorante, compuestos fenólicos totales y actividad antioxidante con respecto al mosto de partida. Esto puede deberse a la presencia de reacciones secundarias (copigmentación, oxidación, condensación…) o a la adhesión de los compuestos en las paredes celulares de las levaduras, que se pierden en el proceso de autolisis de levadura y posterior decantación de estas.
16. Los vinos obtenidos por fermentación espontánea con las levaduras autóctonas de las frutas fueron los que presentaron la menor pérdida de compuestos fenólicos totales, así como de actividad antioxidante. Sin embargo, fueron los vinos procedentes de la inoculación con levaduras aquellos que presentaron la mayor intensidad colorante y concentración de antocianos y flavonoles.
17. De las levaduras estudiadas, la cepa de levadura M05 Mead fue la más apropiada para la fermentación de mostos mezcla de arándanos y uvas, puesto que fue la que obtuvo los vinos con las menores pérdidas de color (absorbancia a 420, 520 y 620 nm), compuestos fenólicos totales y actividad antioxidante, y el mayor contenido tanto en antocianos como en flavonoles.

En el quinto trabajo descrito en el capítulo VII de la Memoria de Tesis Doctoral, titulado ***"Fermentation of blueberry and grape dried mix juices. Color, phenolics compounds and antioxidant activity"***, se llegaron a las siguientes conclusiones:

18. El uso de inóculos de levaduras condujo a un menor tiempo de fermentación y por lo tanto a un menor número de reacciones secundarias, como la menor producción de ácido acético.
19. La deshidratación parcial de una de las frutas que comprende la mezcla de fermentación permitió obtener mostos enriquecidos, siendo el mosto derivado de la mezcla de uva deshidratada y arándano (M2) el que presentó el mayor contenido en azúcar, compuestos fenólicos, color y actividad antioxidante.
20. Los vinos procedentes de la fermentación espontánea de los mostos fueron los que presentaron los peores valores tanto de color, compuestos fenólicos y actividad antioxidante, como las peores valoraciones en el

análisis sensorial debido a un incremento del ácido acético con respecto al resto de vinos.

21. A pesar de partir de mostos con contenidos en azúcar, color, compuestos fenólicos y actividad antioxidante diferentes y, por lo tanto, tiempo de fermentación y maceración también diferentes, los vinos obtenidos con el uso de inóculos de levadura presentaron valores muy parecidos. Destacando aquellos que se han elaborado con la levadura M05 Mead.
22. Desde el punto de vista sensorial, el parámetro mejor valorado fue el color en todos los vinos seguido del aroma en los vinos elaborados a partir de las levaduras X5 y M05. Mientras que el peor valorado fue el parámetro del sabor debido a un exceso de ácido málico. Siendo el vino W2M el mejor puntuado y de mayor calidad sensorial, tanto por catadores expertos como por consumidores habituales de vino.

CONCLUSIONS

In the first work described in Chapter III of the Doctoral Thesis Report, titled ***"Bioactive compounds of chamber-dried blueberries at controlled temperature and wines obtained from them"***, the following conclusions were reached:

1. The drying process increased the absorbances to 420, 520 and 620 nm, as well as the browning index, the contribution of red compounds measured with hue and anthocyanins and tannins contents, due to the greater diffusion of the compounds from the skin to the pulp. However, the content of flavonols, favan-3-oles and vitamin C decreased.
2. The fermentation process produced a decrease in phenolic compounds (anthocyanins, flavonols, flavanols and tannins) and antioxidant activity, while vitamin C remained constant.
3. From the obtained results from sensory analysis, the wines produced that showed residual sugar after fermentation were those that received the best evaluations in flavor and aroma. The color was a parameter positively evaluated in all wines.
4. The wine fermented up to 14 % v/v of alcohol was the best rated wine in sensory analysis, and the one that presented the highest phenolic compound contents and antioxidant activity.

In the second work described in Chapter IV of the Doctoral Thesis Report, entitled ***"The influence of berry perforation on grape drying kinetic and total phenolic compounds"***, *next* conclusions were reached:

5. The increased drying temperature reduced the process time by up to 70 % at 50 ºC compared to the drying time at 30 ºC.
6. The damage caused to the berry by removing the pedicel promoted the diffusion of the water and allowed to reduce the drying time by up to 50% compared to dehydrated whole grapes.
7. The model of *Midilli et al.* (MR = a exp(-kt) + bt) showed the best fit to describe the drying characteristics of whole grapes. nevertheless, for punched grapes, the model of *Two term* (MR = a exp(-k0t) + b exp(-k1t)) was the one that presented the best fitting for temperatures of 30 and 40 ºC and the model of *Aproximation of diffusion* (MR = a exp(-kt) + (1 a) exp(-kbt)) for the temperature of 50 ºC.

8. The effective diffusivity coefficient manifested the diffusion of water, and it was shown that it was higher as the drying temperature was increased, as well as with the perforation of the grape. In addition, it was found that the energy required to remove a mol of water from the fruit (activation energy) was higher for whole grapes (56.49 kJ/mol) than for perforated grapes (54.43 kJ/mol).
9. The phenolic compounds content and the antioxidant activity of the grapes increased after the drying process with respect to the starting grapes, due to the preconcentration effect of the compounds by the loss of water and the greater diffusion of the same ones from the skins to the pulp. Whole grapes had the highest values and therefore a higher thermal stability of phenolic compounds during drying process.

In the third work described in chapter V of the Doctoral Thesis Report, entitled ***"Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (Vaccinium corymbosum)"***, the conclusions reached are shown below:

10. The drying time was reduced with the increase of temperature up to 56% in the case of drying at 50 ºC compared to 30 ºC.
11. The *Page* model (MR = exp(-ktn)) was the best predicting the moisture changes in the drying process at 30 and 50 ºC, while for drying at 40 ºC the best fit was presented by the Approximation of diffusion model (MR = a exp(-kt) + (1-a) exp(-kbt)).
12. The effective diffusivity coefficient increased with temperature, indicating that the temperature facilitated the diffusion of water from the fruit.
13. Although all blueberries were dried until they lost 50 % of the initial moisture, and therefore had the same concentration factor due to evaporation of water, differences were observed between the drying processes. Drying at 50 ºC showed the highest absorbances at 420, 520 and 620 nm, the highest content of phenolic compounds and the highest antioxidant activity measured by both, the ABTS and DPPH assays.

In the fourth work described in Chapter VI of the Doctoral Thesis Report, entitled ***"Phenolic compounds, antioxidant activity and color in the fermentation of blueberry and grape juice with different yeasts"***, the conclusions shown beneath were reached:

14. The use of yeast inoculums allowed to reduce the fermentation time, avoiding the latency phase of yeast growth and avoiding possible secondary reactions.
15. The fermentation process without maceration obtained wines with a loss of color intensity, total phenolic compounds and antioxidant activity with respect to the starting must. This could be due to the presence of secondary reactions (copigmentation, oxidation, condensation…) or the adhesion of compounds in the cell walls of yeasts, which are lost in the process of yeast autolysis and subsequent decantation of yeasts.
16. The wines obtained by spontaneous fermentation with the endogenous yeasts of the fruits were those that presented the least loss of total phenolic compounds, as well as antioxidant activity. However, the wines obtained from the inoculation with yeasts were those that presented the highest color intensity and concentration of anthocyanins and flavonols.
17. Among the yeasts studied, the M05 Mead yeast strain was the most appropriate for the fermentation of mixed blueberries and grapes musts, since it was the one that obtained the wines with the least loss of color (absorbance at 420, 520 and 620 nm), total phenolic compounds and antioxidant activity, and the highest content in both, anthocyanins and flavonols.

In the fifth work described in Chapter VII of the Doctoral Thesis Report, entitled ***"Fermentation of blueberry and grape dried mix juices. Color, phenolics compounds and antioxidant activity"***, the following conclusions were reached:

18. The use of yeast inoculums led to a shorter fermentation time and therefore a lower number of secondary reactions, such as lower production of acetic acid.
19. The partial dehydration of one of the fruits of the fermentation mixture allowed to obtain fortified musts. The must derived from the mixture of dried grapes and undried blueberry (M2) having the highest sugar content, phenolic compounds, color and antioxidant activity.
20. The wines from the spontaneous fermentation of the musts are those that presented the worst values of color, phenolic compounds and antioxidant activity, as the worst evaluations in sensory analysis due to an increase in acetic acid compared to the other wines.
21. Although starting musts had different sugar contents, color, phenolic compounds and antioxidant activity, and therefore, also different

fermentation and maceration time, wines obtained with the use of yeast inoculums showed very similar values. Highlighting those that were made with the yeast M05 Mead.

22. From the sensory point of view, the highest rated parameter was the color in all wines followed by the aroma in the wines made from X5 and M05 yeasts. While the worst rated parameter was the flavor due to an excess that of malic acid. The W2M wine is the best rated and that of higher sensory quality, both by expert tasters and by regular wine consumers.

CAPÍTULO IX

RESUMEN / SUMMARY

RESUMEN

La investigación que compone la presente Memoria de Tesis doctoral forma parte de las principales líneas de investigación del grupo de investigación AGR-270 Fruit Processing de la Universidad de Córdoba, asumiendo como objetivo general la elaboración de bebidas alcohólicas a partir de zumos enriquecidos de frutas.

En el primer trabajo descrito en el capítulo III de la Memoria de Tesis Doctoral, titulado **"*Bioactive compounds of chamber-dried blueberries at controlled temperature and wines obtained from them*"**, se ha estudiado la influencia del secado de arándanos de la variedad Star a 40 ºC, con el objetivo de aumentar el contenido en azúcar de los frutos y poder obtener vinos semi-dulces, dulces y secos con un contenido alcohólico similar a los vinos de uva comerciales. Para ello, se elaboraron tres tipos de vinos, un vino seco, uno semidulce y uno dulce, con diferentes tiempos de fermentación y, por lo tanto, diferentes contenidos de azúcares residuales. En primer lugar, se demostró que tras el proceso de secado aumentaron tanto el contenido de antocianos como el de taninos totales debido al efecto de deshidratación del fruto y a la mayor difusión de los compuestos de la piel a la pulpa; pero en cambio, se perdieron flavonoles, flavanoles y vitamina C que resultaron más sensibles a la temperatura. En segundo lugar, en el proceso de fermentación hubo una pérdida de compuestos fenólicos (antocianos, flavonoles, flavanoles y taninos) y actividad antioxidante con respecto al mosto de partida. En el análisis sensorial los vinos que tenían un mayor contenido de azúcar residual obtuvieron las mejores puntuaciones, siendo el vino fermentado hasta un 14 % v/v de alcohol el vino mejor valorado y con mayor carga de compuestos fenólicos y actividad antioxidante.

En el segundo trabajo descrito en el capítulo IV de la Memoria de Tesis Doctoral, titulado ***"The influence of berry perforation on grape drying kinetic and total phenolic compounds"***, se ha estudiado el secado en cámara con aire caliente en el rango de temperatura de 30 – 50 ºC, de uva de la variedad Tempranillo con y sin pedicelo, con el objetivo de evaluar la influencia que tiene la perforación de la baya a la hora de establecer modelos predictivos de la cinética de secado y en la composición de compuestos fenólicos y actividad antioxidante de la fruta. En primer lugar, el modelo *Midilli et al.* (MR = a exp(-kt) + bt) fue el modelo que mejor se ajustaba a las

características de secado de las uvas que mantienen el pedicelo, mientras que en las uvas perforadas el modelo *Two term* ($MR = a \exp(-k_0 t) + b \exp(-k_1 t)$) fue el que presenta el mejor ajuste para las temperaturas de 30 y 40 ºC, y el modelo de *Approximation of diffusion* ($MR = a \exp(-kt) + (1-a) \exp(-kbt)$) para la temperatura de 50 ºC. El secado de la baya perforada permitió una mayor difusión del agua y, por lo tanto, presentó una mayor velocidad de secado. En todas las temperaturas la baya perforada tuvo un mayor coeficiente de difusividad y, además, la energía de activación para las bayas enteras fue de 56.49 kJ/mol mientras que para las perforadas fue de 54.43 kJ/mol, lo que indica que estas últimas necesitan menos energía para eliminar el agua de la fruta. Las uvas perforadas fueron las que tenían mayor velocidad de secado, pero fueron las bayas enteras las que obtuvieron la mayor concentración en compuestos fenólicos y actividad antioxidante.

En el tercer trabajo descrito en el capítulo V de la Memoria de Tesis Doctoral, titulado ***"Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (Vaccinium corymbosum)"***, se ha estudiado el secado convectivo de arándanos de la variedad Ventura hasta la pérdida del 50 % de su humedad inicial en una cámara con aire caliente, en el rango de temperatura de 30-50 ºC, con el objetivo de obtener zumos de arándanos enriquecidos en compuestos fenólicos y actividad antioxidante. Para poder predecir cambios en el contenido de humedad frente al tiempo de secado, se ajustaron los secados a diferentes modelos, siendo el modelo de *Page* ($MR = \exp(-kt^n)$) el que mejor se ajustó a las características de secado a 30 y 50 ºC, mientras que para el secado a 40 ºC el mejor modelo fue *Approximation of diffusion* ($MR = a \exp(-kt) + (1-a) \exp(-kbt)$). Además, se calculó el coeficiente de difusividad efectiva, que fue mayor conforme aumentó la temperatura y la energía necesaria para eliminar un mol de agua que fue de 23.13 kJ/mol. Estudiada la cinética de secado, se analizaron los zumos obtenidos del prensado de los arándanos secos, de donde se pudo establecer que conforme aumentaba la temperatura aumentaron tanto el color (absorbancia a 420, 520 y 620 nm), como el contenido en compuestos fenólicos totales, la concentración de antocianos y la actividad antioxidante, medida por los ensayos ABTS y DPPH. Siendo, por lo tanto, el secado de arándanos a 50 ºC el más efectivo eliminando agua y el que obtuvo el zumo de arándanos más enriquecido.

En el cuarto trabajo descrito en el capítulo VI de la Memoria de Tesis Doctoral, titulado ***"Phenolic compounds, antioxidant activity and color in the fermentation of blueberry and grape juice with different yeasts"***, se ha estudiado la fermentación de mostos de arándano y uva mezclados con una proporción 1:1 con 4 cepas de levaduras diferentes, con el objetivo de evaluar qué levaduras son las que mantienen el mayor contenido en compuestos fenólicos, color y actividad antioxidante. Los mostos fueron fermentados con cuatro tipos de cepas, de las cuales 3 eran levaduras comerciales QA23, Elegance MP061 y M05 Mead; y una aislada de mostos parcialmente fermentados de uva Pedro Ximenez, X5 (CECT131015). Además del uso de estas cepas de levaduras se utilizó como control la fermentación espontánea de los vinos con las levaduras autóctonas de las frutas. En primer lugar, se demostró que el uso de inóculos de levadura reducía el tiempo de fermentación evitando la fase de latencia donde la levadura se adapta al medio de fermentación de las dos frutas. De los vinos obtenidos, fue el vino control el que menos compuestos fenólicos totales perdió y, por ende, menos actividad antioxidante. En cambio, los vinos obtenidos de la fermentación con inóculos presentaron una menor pérdida de color (absorbancias a 420, 520 y 620 nm) y un mayor contenido en antocianos y flavonoles. La levadura M05 Mead fue la que obtuvo las menores pérdidas de color, compuestos fenólicos totales y actividad antioxidante, y la mayor concentración tanto de antocianos como de flavonoles de las levaduras utilizadas. Por lo tanto, la levadura más apropiada para la vinificación de mostos mezcla de arándanos y uvas es M05 Mead.

En el quinto trabajo descrito en el capítulo VII de la Memoria de Tesis Doctoral, titulado ***"Fermentation of blueberry and grape dried mix juices. Color, phenolics compounds and antioxidant activity"***, se ha estudiado la fermentación de mostos procedentes de la mezcla de arándano y uva 1:1, tras la deshidratación de una de las frutas de la mezcla, con el objetivo de obtener mostos enriquecidos en azúcar, compuestos fenólicos y actividad antioxidante. El proceso de secado se llevó a cabo en una cámara de secado por aire a 50 ºC, las frutas se deshidrataron hasta que perdieron el 50 % de la humedad inicial. Tras esto, se procedió a la mezcla de frutas y al prensado, de donde salieron dos mostos diferentes, uno procedente de uva y arándano deshidratado (M1) y otro procedente de arándano y uva deshidratada (M2). Estos mostos junto con parte de los restos sólidos del prensado se fermentaron a 25 ºC con las levaduras propias de las frutas (fermentación espontánea), con una levadura comercial (M05 Mead) y una levadura aislada de mostos de uva

Pedro Ximenez (X5 CECT131015). Los vinos obtenidos de la fermentación espontánea son aquellos que presentaron los peores valores tanto de color, compuestos fenólicos y actividad antioxidante, así como las peores puntuaciones en el análisis sensorial. A pesar de que los mostos de partida (M1 y M2) presentaron diferentes contenidos en azúcar, y por lo tanto tiempos de fermentación y maceración diferentes, los vinos elaborados con la ayuda de preinóculos presentaron valores de compuestos fenólicos y actividad antioxidante muy similares, destacando aquellos que fueron elaborados con la levadura comercial M05 Mead. Siendo el vino W2M el mejor puntuado y de mayor calidad sensorial, tanto por catadores expertos como por consumidores habituales de vino.

SUMMARY

The research that composes the present Doctoral Thesis Report is part of the main lines of research from AGR-270 Fruit Processing research group of the University of Córdoba, assuming as a general objective the production of alcoholic beverages from enriched fruit juices.

In the first work described in Chapter III of the Doctoral Thesis Report, entitled ***"Bioactive compounds of chamber-dried blueberries at controlled temperature and wines obtained from them"***, the influence of drying blueberries of the variety Star at 40 ºC has been studied with the aim of increasing the sugar content of the fruits to obtain semi-sweet, sweet and dry wines with similar alcoholic content to commercial grape wines. For this purpose, three types of wines were produced, one dry, one semi-sweet and one sweet, with different fermentation times and, therefore, different residual sugar content. Firstly, it was shown that after drying process the anthocyanin and the total tannin contents increased due to the dehydration effect of the fruit and a greater diffusion of the compounds from the skin to the pulp, was achieved contrarily. Flavonols, flavanols and vitamin C were lost because they were more sensitive to temperature. Secondly, in the fermentation process there was a loss of phenolic compounds (anthocyanins, flavonols, flavanols and tannins) and antioxidant activity with respect to the starting must. In sensory analysis, wines with a higher residual sugar content obtained the best scores, among which fermented wine up to 14 % v/v of alcohol was the best-valued wine and had the highest concentration of phenolic compounds and antioxidant activity.

In the second work described in Chapter IV of the Doctoral Thesis Report, entitled ***"The influence of berry perforation on grape drying kinetic and total phenolic compounds"***, chamber-drying process in temperature range of 30 – 50 ºC in Tempranillo grapes with and without pedicel has been studied, to evaluate the influence of berry perforation in establishing predictive models of drying kinetics and in the composition of phenolic compounds and antioxidant activity of the fruit. Firstly, the model *Midilli et al*. ($MR = a \exp(-kt) + bt$) was the best fit of the drying characteristics of the grapes maintaining pedicel, while in the perforated grapes the model *Two term* ($MR = a \exp(-k0t) + b \exp(-k1t)$) was the best fit for 30 and 40 ºC and the *Approximation of diffusion* ($MR = a \exp(-kt) + (1-a) \exp(-kbt)$) model for the

50 ºC of temperature. The drying of punched berries allowed a greater diffusion of water and, therefore, presented a higher drying speed. In all temperatures, the punched berries had a higher coefficient of diffusivity and, in addition, the activation energy for whole berries was 56.49 kJ/mol while for punched berries was 54.43 kJ/mol, indicating that the latter need less energy to remove water from the fruit. The punched grapes were the ones having the highest drying speed, but whole berries obtained the highest concentration in phenolic compounds and antioxidant activity.

In the third work described in Chapter V of the Doctoral Thesis Report, entitled ***"Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (Vaccinium corymbosum)"***, convective drying of blueberries of variety Ventura up to the loss of 50 % of its initial humidity in a hot air chamber has been studied in a temperature range of 30 – 50 ºC. The aim was to obtain blueberry juices enriched in phenolic compounds and antioxidant activity. In order to predict changes in moisture content over drying time, the drying processes were adjusted to different models. Being *Page* model (MR = exp(-ktn)) the best fit for drying characteristics at 30 and 50 ºC, while for drying at 40 ºC the best model was *Approximation of diffusion* (MR = a exp(-kt) + (1-a) exp(-kbt)). In addition, the effective diffusivity coefficient was calculated, which was higher as the temperature increased and energy needed to remove a mole of water resulted 23.13 kJ/mol. After studying the drying kinetics, the juices obtained from the pressing of dried blueberries were analyzed. From results could be established that as the temperature increased so did the color (absorbance at 420, 520 and 620 nm), the content of total phenolic compounds, anthocyanin concentration and antioxidant activity, measured by the ABTS and DPPH assay. Therefore, the drying of blueberries at 50 ºC was the most effective removing water and obtained the most enriched blueberry juice.

In the fourth paper described in Chapter VI of the Doctoral Thesis Report, entitled ***"A Phenolic compounds, antioxidant activity and color in the fermentation of blueberry and grape juice with different yeasts"***, fermentation of blueberry and grape mixed musts in a 1:1 ratio with 4 different yeast strains has been studied. Searching the aim of evaluate that yeasts are those which maintain the highest phenolic compounds content, color and antioxidant activity. The musts were fermented with four types of strains, of which three were commercial yeasts QA23, Elegance MP061, M05 Mead and one isolated from partially fermented musts of Pedro Ximenez, X5

(CECT131015). In addition, the use of these yeast strains was used to control the spontaneous fermentation of the wines with the endigenous yeasts of the fruits. First, it was shown that the use of yeast inoculums reduced the fermentation time avoiding the latency phase where the yeast adapts to the fermentation medium of both fruits. Among the wines obtained, the control wine was the one losing the least total phenolic compounds and, therefore, less antioxidant activity. However, wines obtained from fermentation with inoculum showed the least color loss (absorbances at 420, 520 and 620 nm) and a higher content of anthocyanins and flavonols. In fact, the M05 Mead yeast obtained the lowest loss of color, total phenolic compounds and antioxidant activity; and the highest concentration of anthocyanins and flavonols. Therefore, the most appropriate yeast for the winemaking of mixed blueberries and grapes musts.

In the fifth paper described in Chapter VII of the Doctoral Thesis Report, entitled ***"Fermentation of blueberry and grape dried mix juices. Color, phenolics compounds and antioxidant activity"***, fermentation of must from the mixture of blueberry and grape 1:1 has been studied, after dehydration of one of the fruits of the mixture, with the aim of obtaining musts enriched in sugar, phenolic compounds and antioxidant activity. The drying process was carried out in an air-drying chamber at 50 ºC, the fruits were dehydrated until they lost 50 % of the initial humidity. This was followed by the mixing of fruits and pressing, from which two different musts were obtained, one from dried grapes and undried blueberries (M1) and the other from dried blueberries and undried grapes (M2). These musts with part of the solid remains of the pressing were fermented at 25 ºC with endogenous yeasts (spontaneous fermentation), with a commercial yeast (M05 Mead) and an isolated yeast from grape musts Pedro Ximenez (X5 CECT131015). The wines obtained from the spontaneous fermentation are those which presented the worst values of color, phenolic compounds and antioxidant activity, as well as the worst scores in sensory analysis. Although the starting musts (M1 and M2) had different sugar contents and, therefore, different fermentation and maceration times, wines made with the help of pre-inoculums had very similar values of phenolic compounds and antioxidant activity, highlighting those that were made with commercial yeast M05 Mead. Particularly, W2M wine was the best rated and that of higher sensory quality, after evaluation by expert tasters and regular wine consumers.

CAPÍTULO X

INDICIOS DE CALIDAD

Clave	Artículo
Título	Bioactive compounds of chamber-dried blueberries at controlled temperature and wines obtained from them
Autores	Juan Martin-Gomez, M. Angeles Varo, Julieta Merida, Maria P. Serratosa
Nombre de la revista	Journal of Chemistry
Año, volumen, páginas	2017, 2017, 1-8
Editorial	HINDAWI LTD
Revista incluida en el Journal Citation Reports (JCR)	Sí
Índice de impacto (2017)	1.726
Categorías	Chemistry, multidisciplinary
Lugar que ocupa la revista en las categorías (2017)	97/171
Cuartil	Q3

Clave	Artículo
Título	The influence of berry perforation on grape drying kinetic and total phenolic compounds
Autores	Juan Martin-Gomez, M. Angeles Varo, Julieta Merida, Maria P. Serratosa
Nombre de la revista	Journal of the Science of Food and Agriculture
Año, volumen, páginas	2019, 99(9), 4260-4266
Editorial	Wiley
Revista incluida en el Journal Citation Reports (JCR)	Sí
Índice de impacto (2018)	2.422
Categorías	Agriculture, multidisciplinary – Chemistry, applied – Food science & technology
Lugar que ocupa la revista en las categorías (2018)	9/56 – 23/71 – 43/135
Cuartil	Q1 – Q2 – Q2

Clave	Artículo
Título	Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (Vaccinium corymbosum)
Autores	Juan Martin-Gomez, M. Angeles Varo, Julieta Merida, Maria P. Serratosa
Nombre de la revista	LWT- Food science and technology
Año, volumen, páginas	2020, 120
Editorial	ELSEVIER SCIENCE BV
Revista incluida en el Journal Citation Reports (JCR)	Sí
Índice de impacto (2018)	3.714
Categorías	Food science & Technology
Lugar que ocupa la revista en las categorías (2018)	23/135
Cuartil	Q1

CAPÍTULO XI

OTRAS APORTACIONES CIENTÍFICAS

PUBLICACIONES CIENTÍFICAS

Co-autores: Jesús Hidalgo-Carrillo, Juan Martín-Gómez, Julia Morales, Juan Carlos Espejo, Francisco José Urbano and Alberto Marinas
Título del artículo: Hydrogen photo-production from glicerol using nickel-doped TiO2 catalysts: effect of catalyst pre-treatment
Título de la revista: energies
Volumen: 12 **Año:** 2019
Índice de impacto (2018): 2.676
Índice relativo que tenga dentro de su categoría: 48/97 Energy & fuels
Fuente: Journal Citation Reports

CAPÍTULOS DE LIBROS

Co-autores: M.A. Varo, J. Martín, A. Hurtado, A. Márquez, M. P. Serratosa
Título del capítulo: Extracción de compuestos bioactivos de residuos de uvas tintas
Título del libro: XXXVI Jornadas de viticultura y enología. Tierra de Barros
Editorial: Centro Universitario Santa Ana
Lugar de edición: Almendralejo Año de edición: 2014 Páginas: 55
ISBN: 978-84-7930-103-1

Co-autores: M.A. Varo, J. Martín, J. Hungría, A. Márquez, L. Moyano, M. P. Serratosa
Título del capítulo: Determinación de compuestos bioactivos en frutos rojos
Título del libro: VIII Congreso CYTA/CESIA. Conocimientos para impulsar una evolución inteligente del sector alimentario
Lugar de edición: Badajoz **Año de edición:** 2015 **Páginas:** 103
ISBN: 978-84-606-6881-7

Co-autores: J. Martín-Gómez, M. A. Varo, M. P. Serratosa, J. Mérida
Título del capítulo: Estudio del proceso de secado de arándanos
Título del libro: I Congreso veterinaria y ciencia y tecnología de los alimentos
Editorial: Universidad de Córdoba
Lugar de edición: Córdoba **Año de edición:** 2016 **Páginas:** 17

ISBN: 978-84-608-5864-5

Co-autores: J. Martín-Gómez, M. A. Varo, M. P. Serratosa, J. Mérida
Título del capítulo: The influence of fermentation time in the composition of blueberry fermented beverages
Título del libro: III National and II international student congress of food science and technology
Editorial: Asociación Valenciana de Jóvenes Estudiantes de Ciencia y Tecnología de los Alimentos (AVECTA)
Lugar de edición: Valencia **Año de edición:** 2016 **Páginas:** 87
ISBN: 2341-2240

Co-autores: M. A. Varo, J. Martín-Gómez, M. P. Serratosa, J. Mérida
Título del capítulo: Effect of sulphur dioxide as preservative in the bioactive compounds and color of blueberry fermented beverages
Título del libro: III National and II international student congress of food science and technology
Editorial: Asociación Valenciana de Jóvenes Estudiantes de Ciencia y Tecnología de los Alimentos (AVECTA)
Lugar de edición: Valencia **Año de edición:** 2016 **Páginas:** 86
ISBN: 2341-2240

Co-autores: M. A. Varo, J. Martín-Gómez, L. Nuñez-Cárdenas, M. P. Serratosa, J. Mérida
Título del capítulo: Influencia del tiempo del fermentación en la composición de compuestos antócianos, vitamina C y actividad antioxidante de vinos de arándanos
Título del libro: IX Congreso CyTA/CESIA
Editorial: Universidad Rey Juan Carlos
Lugar de edición: Madrid **Año de edición:** 2017 **Páginas:** 80
ISBN: 978-84-608-4658-1

Co-autores: J. Martín-Gómez, M. A. Varo, M. P. Serratosa, J. Mérida
Título del capítulo: ID23532- Fermented beverages from dried blueberries
Título del libro: 9º Congreso internacional de Química de la ANQUE. Alimentos y bebidas
Editorial: ANQUE
Lugar de edición: Murcia **Año de edición:** 2018 **Páginas:** 47

ISBN: 978-84-09-02880-1

Co-autores: M. A. Varo, J. Martín-Gómez, M. P. Serratosa, J. Mérida
Título del capítulo: ID23533- Antioxidant activity and bioactive compounds determined by high performance liquid chromatography / mass spectrometry, from blueberry (Vaccinium Myrtillus L.) juice by-products
Título del libro: 9º Congreso internacional de Química de la ANQUE. Alimentos y bebidas
Editorial: ANQUE
Lugar de edición: Murcia **Año de edición:** 2018 **Páginas:** 63
ISBN: 978-84-09-02880-1

Co-autores: J. Martín-Gómez, F. J. López-Tenllado, V. Montes, J. Morales-Roldan, A. Marinas, F. J. Urbano
Título del capítulo: Facilitando la síntesis de fotocatalizadores para fotoproducción de H2
Título del libro: Catálisis para un futuro sostenible
Editorial: SECAT
Lugar de edición: Valencia **Año de edición:** 2018 **Páginas:** 92
ISBN: 978-84-09-03041-5

Co-autores: Jesús Hidalgo-Carrillo, Francisco J. López-Tenllado, Elena Sanchez-Lopez, Juan Martín-Gómez, Alberto Marinas, Francisco J. Urbano
Título del capítulo: Obtención de hidrógeno por vía fotocatalítica a partir de alcoholes provenientes de la biomasa
Título del libro: XXVI Congresso ibero-americano de catálise
Editorial: Sociedade Portuguesa de Química
Lugar de edición: Coimbra **Año de edición:** 2018 **Páginas:** 1953 – 1957
ISBN: 978-989-8124-23-4

Co-autores: J. Martín-Gómez, F. J. López-Tenllado, V. Montes, J. Hidalgo-Carrillo, J. C. Escamilla, A. Marinas, F. J. Urbano
Título del capítulo: Fotoproducción de H2 por mezcla física de CuO y TiO2
Título del libro: NANOUCO VII. Encuentro sobre nanociencia y nanotecnología
Editorial: IUNAN
Lugar de edición: Córdoba **Año de edición:** 2019 **Páginas:** 73

ISBN: 978-84-09-14458-7

Co-autores: Juan Martín Gómez, Francisco Javier López Tenllado, Vicente Montes Jiménez, Jesús Hidalgo Carrillo, Alberto Marinas Aramendía, Francisco José Urbano Navarro
Título del capítulo: Mezcla física de CuO-TiO2 como catalizador en la fotoproducción de H2
Título del libro: Catálisis para el futuro: avances en estructuras, procesos y aplicaciones
Editorial: UCO press
Lugar de edición: Córdoba **Año de edición:** 2019 **Páginas:** 449-450
ISBN: 978-84-9927-454-6

Co-autores: Juan Carlos Escamilla Mejía, Juan Martín Gómez, Vicente Montes Jiménez, Jesus Hidalgo carrillo, Alberto Marinas Aramendía, Francisco José Urbano Navarro
Título del capítulo: Producción de H2 mediante foto-reformado de glicerol utilizando catalizadores TiO2/CA
Título del libro: Catálisis para el futuro: avances en estructuras, procesos y aplicaciones
Editorial: UCO press
Lugar de edición: Córdoba **Año de edición:** 2019 **Páginas:** 447-448
ISBN: 978-84-9927-454-6

CONTRIBUCIONES A CONGRESOS

Comunicaciones póster en congresos nacionales

Co-autores: M.A. Varo, J. Martín, J. Hungría, A. Márquez, L. Moyano, M. P. Serratosa
Lugar: Badajoz **Fecha:** 08/04/2015 – 10/04/2015
Título del Congreso: VIII Congreso CYTA/CESIA. Conocimientos para impulsar una evolución inteligente del sector alimentario
Título del póster: Determinación de compuestos bioactivos en frutos rojos

Autor: Juan Martín Gómez
Lugar: Córdoba **Fecha:** 18/01/2018 – 19/01/2018
Título del Congreso: VI Congreso científico de investigadores en formación de la Universidad de Córdoba

Título del póster: Procesos de deshidratación de frutas. Cinética y efecto sobre la composición en compuestos bioactivos y calidad organoléptica de las frutas

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Co-autores: J. Martín-Gómez, F. J. López-Tenllado, V. Montes, J. Hidalgo-Carrillo, J. C. Escamilla, A. Marinas, F. J. Urbano
Lugar: Córdoba **Fecha:** 21/01/2019 – 22/01/2019
Título del Congreso: NANOUCO VII. Encuentro sobre nanociencia y nanotecnología de investigadores andaluces
Título del póster: Fotoproducción de H2 por mezcla física de CuO y TiO2

Co-autores: Juan Martín Gómez, Francisco Javier López Tenllado, Vicente Montes Jiménez, Jesús Hidalgo Carrillo, Alberto Marinas Aramendía, Francisco José Urbano Navarro
Lugar: Córdoba **Fecha:** 24/06/2019 – 26/06/2019
Título del Congreso: Catálisis para el futuro: avances en estructuras, procesos y aplicaciones
Título del póster: Mezcla física de CuO-TiO2 como catalizador en la fotoproducción de H2

Co-autores: Juan Carlos Escamilla Mejía, Juan Martín Gómez, Vicente Montes Jiménez, Jesus Hidalgo carrillo, Alberto Marinas Aramendía, Francisco José Urbano Navarro
Lugar: Córdoba **Fecha:** 24/06/2019 – 26/06/2019
Título del Congreso: Catálisis para el futuro: avances en estructuras, procesos y aplicaciones
Título del póster: Producción de H2 mediante foto-reformado de glicerol utilizando catalizadores TiO2/CA

Comunicaciones orales en congresos nacionales

Co-autores: M.A. Varo, J. Martín, A. Hurtado, A. Márquez, M. P. Serratosa
Lugar: Almendralejo **Fecha:** 07/05/2014
Título del Congreso: XXXVI Jornadas de viticultura y enología. Tierra de Barros
Título de la comunicación: Extracción de compuestos bioactivos de residuos de uvas tintas
Presentada por Dª.: M.A. Varo

Co-autores: J. Martín-Gómez, M. A. Varo, M. P. Serratosa, J. Mérida

Lugar: Córdoba **Fecha:** 12/02/2016
Título del Congreso: I Congreso veterinaria y ciencia y tecnología de los alimentos
Título de la comunicación: Estudio del proceso de secado de arándanos
Presentada por D.: J. Martín-Gómez

Co-autores: M. A. Varo, J. Martín-Gómez, L. Nuñez-Cárdenas, M. P. Serratosa, J. Mérida
Lugar: Madrid **Fecha:** 16/05/2017 – 19/05/2017
Título del Congreso: IX Congreso CyTA/CESIA. Ayer, hoy y mañana de la ciencia y tecnología de los alimentos
Título de la comunicación: Influencia del tiempo del fermentación en la composición de compuestos antócianos, vitamina C y actividad antioxidante de vinos de arándanos
Presentada por D.: J. Martín-Gómez

Co-autores: J. Martín-Gómez, F. J. López-Tenllado, V. Montes, J. Morales-Roldan, A. Marinas, F. J. Urbano
Lugar: Valencia **Fecha:** 25/06/2018 – 27/06/2018
Título del Congreso: III encuentro de jóvenes investigadores de la sociedad española de catálisis
Título de la comunicación: Facilitando la síntesis de fotocatalizadores para fotoproducción de H2
Presentada por D.: J. Martín-Gómez

Comunicaciones póster en congresos internacionales

Co-autores: J. Martín-Gómez, M. A. Varo, M. P. Serratosa, J. Mérida
Lugar: Valencia **Fecha:** 03/03/2016 – 04/03/2016
Título del Congreso: III National and II international student congress of food science and technology
Título del póster: The influence of fermentation time in the composition of blueberry fermented beverages

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Co-autores: M. A. Varo, J. Martín-Gómez, M. P. Serratosa, J. Mérida
Lugar: Valencia **Fecha:** 03/03/2016 – 04/03/2016
Título del Congreso: III National and II international student congress of food science and technology

Título del póster: Effect of sulphur dioxide as preservative in the bioactive compounds and color of blueberry fermented beverages

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Co-autores: J. Martín-Gómez, M. A. Varo, J. Mérida, M. P. Serratosa
Lugar: Córdoba **Fecha:** 26/09/2017 – 29/09/2017
Título del Congreso: 10th international conference on predictive modelling in food, ICPMF10
Título del póster: Modellign and study of drying variables in the drying of grape without pedicel

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Co-autores: Lourdes Moyano Cañete, Juan Martín Gómez, María Pérez Serratosa, Lázaro Nuñez Cárdenas, M. Ángeles varo Santos
Lugar: Córdoba **Fecha:** 26/09/2017 – 29/09/2017
Título del Congreso: 10th international conference on predictive modelling in food, ICPMF10
Título del póster: Identification of aroma and color metabolites to assay genes involved in the organoleptic quality os strawberry fruit

Comunicaciones orales en congresos internacionales

Co-autores: J. Martín-Gómez, M. A. Varo, M. P. Serratosa, J. Mérida
Lugar: Murcia **Fecha:** 17/06/2018 – 20/06/2018
Título del Congreso: 9º Congreso internacional de Química de la ANQUE. Alimentos y bebidas
Título de la comunicación: Fermented beverages from dried blueberries
Presentada por D.: J. Martín-Gómez

Co-autores: M. A. Varo, J. Martín-Gómez, M. P. Serratosa, J. Mérida
Lugar: Murcia **Fecha:** 17/06/2018 – 20/06/2018
Título del Congreso: 9º Congreso internacional de Química de la ANQUE. Alimentos y bebidas
Título de la comunicación: Antioxidant activity and bioactive compounds determined by high performance liquid chromatography / mass spectrometry, from blueberry (Vaccinium Myrtillus L.) juice by-products
Presentada por D.: M. A. Varo

Co-autores: Jesús Hidalgo-Carrillo, Francisco J. López-Tenllado, Elena Sanchez-Lopez, Juan Martín-Gómez, Alberto Marinas, Francisco J. Urbano
Lugar: Coimbra **Fecha:** 09/09/2018 – 14/09/2018
Título del Congreso: XXVI Congresso ibero-americano de catálise

Título de la comunicación: Obtención de hidrógeno por vía fotocatalítica a partir de alcoholes provenientes de la biomasa
Presentada por D.: Alberto Marinas

CAPÍTULO XII

PUBLICACIONES

Hindawi

Journal of Chemistry

Indexed in Science Citation Index Expanded

Journal of Chemistry
Volume 2017, Article ID 1567106, 8 pages
https://doi.org/10.1155/2017/1567106

Research Article

Bioactive Compounds of Chamber-Dried Blueberries at Controlled Temperature and Wines Obtained from Them

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Received 3 April 2017; Revised 8 August 2017; Accepted 14 September 2017; Published 16 October 2017

Academic Editor: Wieslaw Wiczkowski

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Journal of Chemistry
Volume 2017, Article ID 1567106, 8 pages
https://doi.org/10.1155/2017/1567106

Research Article

Bioactive Compounds of Chamber-Dried Blueberries at Controlled Temperature and Wines Obtained from Them

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Received 3 April 2017; Revised 8 August 2017; Accepted 14 September 2017; Published 16 October 2017

Academic Editor: Wieslaw Wiczkowski

The effects of chamber drying under controlled temperature and moisture conditions and fermentation process on blueberry juices to obtain three wines were studied in this work. Drying was carried out with a view to increase the sugar content and obtain wines with an ethanol content similar to a commercial grape wine or to obtain sweet wines. Analyses included color parameters; browning index; and anthocyanin, flavonols, flavan-3-ol derivatives, and tannin concentrations, as well as vitamin C concentration and antioxidant activity. Based on the results, drying increases color and the concentration of anthocyanins and tannins most probably by the effect of dehydration of the berries and diffusion of the colored compounds from the skin to the pulp due to the structural alterations in their skin. In addition, drying decreases flavonols, flavan-3-ol derivatives, and vitamin C concentrations. The browning index, anthocyanins, and tannins decreased with the fermentation time, and vitamin C was constant with the fermentation time. The sensory analysis showed that the wines with the best sensory characteristics were those with residual sugar, partial fermented wines 1 and 2.

1. Introduction

Blueberries, *Vaccinium corymbosum*, are native to eastern North America and southeastern Canada [1]; however, they are extending to Europe, Asia, Africa, and Australia. Blueberries can be consumed as fresh fruits and also in products derived as seasoning products of bakery, pastry filling, juices, yogurt, and so forth [2]. Besides, in the last years, the use of different berries for the elaboration of fermented beverages or wines has spread, principally due to the fact that grapes are not well cultivated in every region of the world. Another type of fruit is used to carry out the fermentation process, with the berries of red fruits being some of the most used due to their phenolic composition, especially in anthocyanins content [3]. Yan et al. [4] have studied the optimal conditions of blueberries juice fermentation, with these conditions being a temperature of 22.65°C and a pH of 3.53. They carried out the fermentation of the blueberry juice adding sucrose, to reach the alcoholic graduation of a standard wine. Nevertheless, other authors have developed direct fermentation on the juice, reaching only 5-6% of alcohol, due to the concentration of sugar [5, 6].

Blueberries are functional food due to their health benefits (e.g., antioxidant, anti-inflammation, neuroprotection, antimetastatic, cardioprotective, antimicrobial, renoprotective, opthalmoprotective, antidiabetic, hepatoprotective, gastroprotective, antiosteoporotic, and antiaging) [7]. These properties are consequent of their phenolic composition [8]. The protective activity is attributed to their antioxidant capacity and free radical scavenger in addition to the ability to inhibit and reduce enzymes [9]. The phenolic compounds of blueberries include anthocyanins (anthocyanidins glycosides) which are in the cell vacuoles in the red fruit skins [10]. They are responsible for the fruit color, between red and blue characteristic of blueberry. Flavonols are a flavonoid family ranging from white to yellow in color depending on their structure, but in red wines they can participate in the color because they are copigments of anthocyanins [11]. Flavan-3-ol derivatives are flavonoid compounds with optical isomerism; (+)-catechin and (−)-epicatechin are the most

representative, and they are found in the seeds and stems of the grapes [12]. Tannins encompass a series of phenolic compounds, including condensed tannins (namely, variably complex polymers of flavan-3-ol) and hydrolysable tannins (ellagitannins and gallotannins, mainly); they are highly astringent and bitter compounds present in seeds of fruits.

The addition of sucrose is the most common method to increase the sugar content of blueberry juices [13–15] to obtain a fermented beverage with a standard ethanol content. Nevertheless, the partial dehydration of the berry can increase the sugar content in a similar way to that made in southern Spain or Italy for the production of sweet wines. Sun-drying is the most traditional drying method, but artificial drying with controlled temperature, relative humidity, and air flow had some advantages [16] such as avoiding losses for the weather (rain) or the attack of insects and fungi. These methods affect the berry's properties such as color, texture, and density [17].

The aim of this work was to evaluate the changes in bioactive compounds, color, and antioxidant activity during the drying process and subsequent fermentation of sugar-rich juices to obtain blueberry wines.

2. Materials and Methods

2.1. Reagents. Hydrochloric acid, metaphosphoric acid, formic acid, acetic acid, methanol, acetonitrile, sodium metabisulphite, potassium chloride, sodium acetate, acid potassium dichromate, ethyl acetate, and potassium dihydrogen phosphate were purchased from Merck (Madrid, Spain). Anthocyanins (malvidin-3-O-galactoside chloride), flavan-3-ol derivative ((+)-catechin, (−)-epicatechin, epigallocatechin gallate, procyanidin B1, and procyanidin B2), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenylpicrylhydrazyl), and DTT (DL-dithiothreitol) were purchased from Sigma-Aldrich Chemical Co. (Madrid, Spain).

2.2. Blueberry Drying. The blueberries used for this study were Star variety *(Vaccinium corymbosum)* and they were harvested in 2015 in Moguer (Huelva, Spain) at the usual state of maturity for commercialization. One portion was crushed for analysis.

Three batches of blueberries were dried in a Frisol Climatronic chamber at a constant temperature of 40°C and an initial relative humidity of 20%. During the drying process, a sample was collected daily and the weight loss and reducing sugar concentration of the blueberries were measured using a refractometer, model Atago Master.

The drying process was finished when the reducing sugar content reached approximately 24.2°Brix. The blueberries were pressed on a vertical press similar to industrial models, obtaining the final juice. The maximum pressure reached in each of the two pressing cycles was 300 bar. The musts were centrifuged at 5000 rpm, filtered, and analyzed in triplicate.

2.3. Fermentation Process. The sweet juice was divided into three batches and was mixed with the solid parts of the berries in a ratio of 1 : 1 (w/v). The mix was added with a yeast inoculum of commercial *Saccharomyces cerevisiae* in a dose recommended by the manufacturer (0.3 g/L). The flasks were immersed in thermostatized water baths at 22 ± 0.5°C [4]. Three wines were elaborated in triplicate from the dried blueberries.

Wine 1. The mix was partially fermented to 6% of ethanol (v/v) and the process was stopped by the addition of wine alcohol up to 17% (v/v).

Wine 2. The mix was partially fermented to 14% of ethanol (v/v) and the process was stopped by the addition of wine alcohol up to 17% (v/v).

Wine 3. The mixture was completely fermented, obtaining a wine with a 17% (v/v) ethanol content.

After fermentation/maceration, the berries were pressed a second time on a vertical press and skin residues were removed from the wine. The resulting wines were centrifuged at 5000 rpm, filtered, and analyzed in triplicate.

2.4. Reducing Sugars. This parameter was determined according to the EEC official methods as described in Regulation 2676/1990 [18].

2.5. Volatile Acidity. Isolation of volatile acids is carried out according to the method of the OIV [19] by steam distillation and rectification of the distillate.

2.6. Spectrophotometric Determinations. Spectrophotometric measurements were made on a PerkinElmer (Waltham, MA) Lambda 25 spectrophotometer, using quartz cells of 1 mm light path. Samples were previously passed through Millipore (Billerica, MA) HA filters of 0.45 μm pore size. All measurements were corrected for a path length of 1 cm.

Absorbances at 420, 520, and 620 nm were measured. Hue indicates the proportion between orange and red colors; $A420$ and $A520$ nm relation was calculated.

2.6.1. Polymeric Pigments Color (PPC). To obtain PPC values, 5 mL of wine was added with 15 mg of $Na_2S_2O_5$; after 45 min, the absorbance at 520 nm was measured. Anthocyanin monomers were immediately decolorized by the excess $Na_2S_2O_5$ added, so the residual color was due to the polymeric forms of the pigments.

2.6.2. Antioxidant Activity. Antioxidant activity was analyzed through the DPPH assay according to Alén-Ruiz et al. [20] with some modifications. The juice and wines were diluted at 1 : 5 with distilled water. A 45 mg/L solution of DPPH (2,2-diphenylpicrylhydrazyl) in methanol was prepared on a daily basis and stored in the dark. An 80 mg/L solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a vitamin E analog, was used as a standard. The analytical procedure was as follows: a 200 μL aliquot of diluted sample was placed in a cell and 3 mL of a 45 mg/L solution of DPPH in methanol was then added. A blank (200 μL diluted sample + 3 mL methanol), a control sample (200 μL of 12% ethanol in water + 3 mL of DPPH solution), and a Trolox standard (200 μL of Trolox solution + 3 mL of

DPPH solution) were also prepared in parallel. Following vigorous stirring, the absorbances at 517 nm of the control sample and blank were measured in a PerkinElmer (Waltham, MA) Lambda 25 spectrophotometer. The sample and the Trolox standard were measured under identical conditions after 120 min of incubation at room temperature. The results were expressed in millimoles of Trolox per liter.

$$\begin{aligned}&\text{Antioxidant activity (mmol TE/L)}\\&\quad=\frac{(0{,}32\cdot A_1\cdot \text{dilution factor})}{A_2},\\&A_1=\text{Absorbance}_{\text{control }(t=0)}-\text{Absorbance}_{\text{sample}},\\&A_2\\&\quad=\text{Absorbance}_{\text{control }(t=0)}-\text{Absorbance}_{\text{standard }(t=120)},\\&\text{Absorbancia}_{\text{sample}}\\&\quad=\text{Absorbancia}_{\text{sample }(t=120)}-\text{Absorbancia}_{\text{blanc }(t=0)}.\end{aligned}\tag{1}$$

2.6.3. Total Anthocyanins. The total monomeric anthocyanin pigment content was measured by the pH differential method described by Lee et al. [21]. This method is based on the change of the maximum of absorbance with a change in pH of the monomeric anthocyanins. Two dilutions at 1 : 10 of juice samples were prepared with pH 1.0 buffer (potassium chloride) and pH 4.5 buffer (sodium acetate) and the absorbance was measured at 500 and 720 nm within 20–50 minutes of preparation. Calculate total anthocyanin concentration, expressed as cyanidin-3-glucoside equivalents, as follows:

$$\begin{aligned}&\text{Total monomeric anthocyanins (mg/L)}\\&\quad=\frac{A\cdot M_W\cdot D\cdot 1000}{\varepsilon\cdot \text{LP}}\\&A=(A_{520}-A_{700})_{\text{pH1}}-(A_{520}-A_{700})_{\text{pH4,5}},\end{aligned}\tag{2}$$

where M_W is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), D is the dilution factor, ε is molar extinction coefficient for cyanidin-3-glucoside (26900 L/mol·cm), and LP is the light path.

2.6.4. Total Tannins. The total tannins were determined by measurement of the absorbance at 550 nm after acid hydrolysis of the samples diluted at 1 : 50 with distilled water and a blank. The resulting absorbance ($A_{\text{sample}} - A_{\text{blank}}$) was multiplied by a factor of 19.33, in order to calculate the total tannin concentration, in g/L.

2.6.5. Ethanol Content. Ethanol content was determined according to Crowell and Ough [22]; to this end, ethanol in the sample was collected by steam and then reacted with acid potassium dichromate. The reaction was spectrophotometrically monitored via the absorbance at 600 nm against a blank on a PerkinElmer Lambda 25 spectrophotometer.

2.7. Flavonols Extraction. A volume of 2 mL of must was passed through a Sep-Pak C18 cartridge, with 900 mg of filling (Long Body Sep-Pak Plus; Waters Associates, Milford, Massachusetts) that was previously activated with 5 mL of pure methanol and washed with aqueous 0.01% (v/v) HCl. The cartridge was successively washed with 10 mL of 0.01% aqueous HCl. The cartridge was eluted with 5 mL of pure ethyl acetate. This collected fraction was evaporated on a rotary evaporator thermostated at 35°C and resolved in 1 mL of pure methanol. The fraction was passed through a filter of 0.45 μm pore size before injection into the HPLC instrument.

2.8. Identification and Quantification of Flavonols by HPLC-DAD. A volume of 50 μL of the sample was injected into an HPLC-DAD instrument (Beckman Coulter System Gold, 168 Detector) and the analyses were carried out on a LiChrospher 100 RP-18 column (250 mm × 4.6 mm, 5 μm), according to the method proposed by Marquez et al. [23]. The identification was carried out by comparing their retention times with those for standards, recording UV-Vis spectra, and calculating the UV absorbance ratios for samples and standards simultaneously coinjected one at a time.

2.9. Identification and Quantification of Flavan-3-ol Derivatives. In the case of identification and quantification of flavan-3-ol derivatives, the samples were diluted 25 times in ultrapure water. The identification and quantification were carried out in an HPLC (Thermo Spectra Physics Series P100) with a fluorescence detector (PerkinElmer Series 200a), on a LiChrospher 100 RP-18 column (250 mm × 4.6 mm, 5 μm), according to the method proposed by Marquez et al. [23].

2.10. Vitamin C. 0.7 mL of 4.5% metaphosphoric acid was added to 0.7 mL of juice, and the mix was centrifuged at 5000 rpm for 10 minutes at 4°C. 1 mL of the mix was added to 0.2 mL of DTT (DL-dithiothreitol) solution and the sample was kept in the dark for 2 hours in order to reduce the dehydroascorbic acid to L-ascorbic acid. After complete conversion, the sample was filtered with a nylon filter of 0.45 μm pore size. Ascorbic acid quantification was performed on an HPLC chromatograph (Thermo Spectra Physics Series P100) coupled to a UV detector (Thermo Finnigan Spectra System UV2000), using a LiChrospher 100 RP-18 column (250 mm × 4.6 mm, 5 μm). KH_2PO_4 (0.2 M at pH = 2.3–2.4) was used as a mobile phase with a flow of 1.0 mL/min for 15 minutes at λ = 243 nm and an injection volume of 20 μL.

2.11. Sensory Analysis of Wines. The wines were assessed for aroma, flavor, and color acceptability in accordance with ISO 8586-1:1993. The tasting room was kept at 20°C and wines were served in coded tasting glasses certified in accordance with ISO 3591:1977. The sixteen tasters were instructed in advance in their task and the rules to be followed and were given a scoring sheet. The evaluation of the quality of wines was made according to ISO 4121:2003, with options of desirable (5-6), acceptable (3-4), and undesirable (1-2).

2.12. Statistical Procedures. The results for all samples were subjected to analysis of variance at the 95.0% confidence level; in addition, homogeneous groups were calculated in

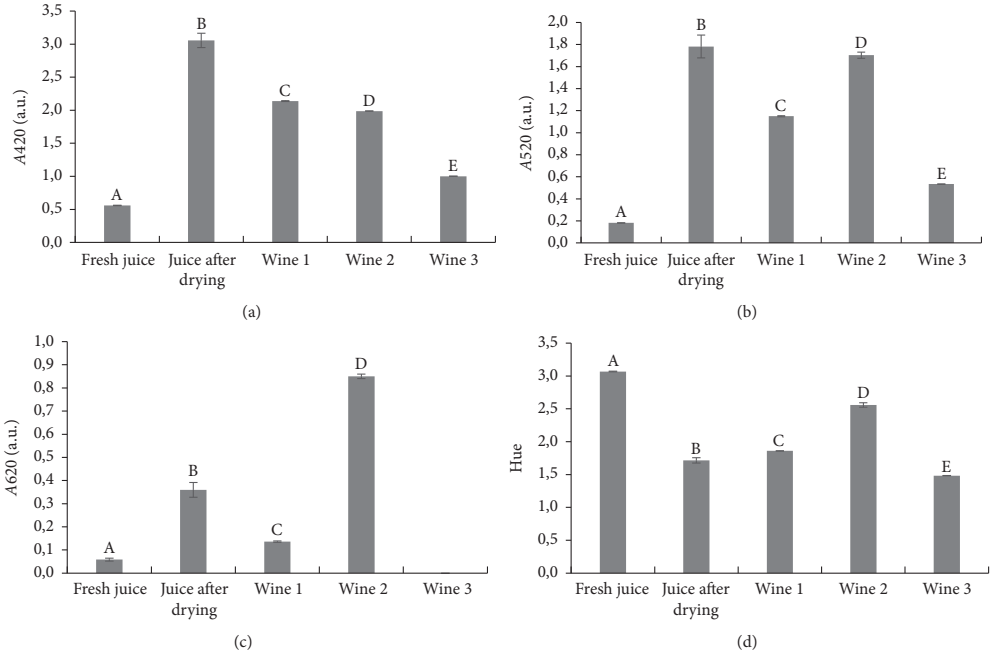

Figure 1: Changes in (a) absorbance at 420 nm (a.u.), (b) absorbance at 520 nm (a.u.), (c) absorbance at 620 nm (a.u.), and (d) hue for blueberry juices and wines. $N = 3$. Columns with different superscript letters are significantly different, $p = 0.05$.

order to establish significant differences between means. The software used was the Statgraphics Computer Package v.5.0 from Statistical Graphics Corp.

3. Results and Discussion

The first stage in the elaboration of blueberry wine was a drying process in order to increase the sugar content to obtain a fermented beverage with an ethanol content similar to a grape wine. The blueberry variety presented a sugar content of 13.2°Brix. This content increased to 24.2°Brix after 48 hours of drying. During the drying process, evaporation of water occurred, causing the concentration of other compounds besides the sugars. A sweet juice was obtained from the dried blueberries and this juice was subjected to a fermentation process obtaining three wines with different sugar contents due to the different times of fermentation. Wine 1 was a sweet wine with 117 g/L of residual sugars, wine 2 was a semisweet wine (28.8 g/L of residual sugars), and wine 3 was a dry wine (0 g/L of residual sugars).

The volatile acidity was measured in order to control the quality of wines. This parameter represents the amount of volatile acids in the wines, acetic acid being the majority. The volatile acidity increased with the fermentation time with values of 5.4, 5.9, and 8.9 meq/L for wine 1, wine 2, and wine 3, respectively. The latter presented a value higher than the limit established in the Official State Bulletin [24] for commercial grape wines.

Color is the first attribute perceived by the consumer in food. The absorbances at 420, 520, and 620 nm as well as the hue were measured and expressed as absorbance units (a.u.). These three absorbances represent the contribution of browning and red and blue compounds, respectively. As can be seen in Figure 1, the absorbances increased in the drying process, so the color intensity of the juice from dried fruits was higher than of the juice from fresh fruits. In relation to wines, the browning index ($A420$) decreased with the fermentation time; wine 3 presented the lowest value. The values of absorbances at 520 and 620 nm were the highest in wine 2; wine 3 presented the lowest values of both absorbances. This may be a result of the adsorption of colored compounds by the cell walls of the yeasts [25]. The hue, expressed as the ratio between absorbances at 420 and 520 nm (Figure 1(d)), decreased in the drying process, although the values were always above value 1, which indicates greater contribution of the brown compounds. In wines, wine 3 (total fermentation) was the beverage with the lowest value.

The polymeric pigments color measured in wines shows that the fermentation process produced a decrease of PPC with time (0.699, 0.517, and 0.373 in wine 1, wine 2, and wine 3, resp.), so the contribution of pigment polymers to color decreases. On the one hand, the high value of wine 1 was due to the concentration resulting from the loss of water by evaporation during the drying process. On the other hand, the maceration with solid parts should increase the values

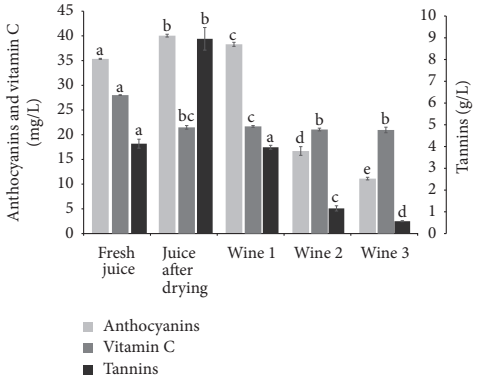

Figure 2: Changes in anthocyanins, vitamin C, and tannins of blueberry juices and wines. $N = 3$. Columns of the same parameter with different superscript letters are significantly different, $p = 0.05$.

Figure 3: Antioxidant activity for blueberry juices and wines. $N = 3$. Columns with different superscript letters are significantly different, $p = 0.05$.

during the fermentation. Nevertheless, these compounds decreased during the maceration, possibly due to other reactions in which they may be involved.

Anthocyanins are polyphenolic pigments which are responsible for the red color of blueberry juices and wines. The drying process increased the concentration of these compounds due to the water evaporation and the diffusion process from skin to juice due to the structural alterations in fruit skin [26]. After the drying, the anthocyanins content was 40 mg/L (Figure 2). In wines, the concentration of monomer anthocyanins decreased with the fermentation time, with wine 3 presenting the lowest concentration. This fact could be due to, on the one hand, the adsorption of anthocyanin by the cell membranes of yeast; as the time of contact with yeasts increases, a reduction of these compounds occurs in the juice [25]. Furthermore, condensation reactions occur between the monomeric anthocyanins and some yeast metabolites resulting in new compounds called pyranoanthocyanins [27] or condensation reactions with other phenolic compounds such as flavonols and flavans [28].

Regarding the concentration of total vitamin C, fresh blueberries showed the highest value (28.1 mg/L) (Figure 2). The drying process produced a decrease in total vitamin C content to 21.5 mg/L. This vitamin C concentration was maintained without significant changes during the fermentation process. Vitamin C is a typical heat sensitive micronutrient, so the decrease during the drying process is due to the temperature [29]. Then, the fermentation process did not affect the content of this vitamin.

Tannins are polymer compounds responsible for the astringency of wines. In wines, certain astringency is appreciable, although if it is excessive it becomes a defect. Drying process raised its content from 4.13 to 8.95 g/L (Figure 2), due mainly to the concentration effect by water evaporation. However, the fermentation process produced a decrease in total tannin values, which was more pronounced as the fermentation time increased. The final values of the wines were 3.97 g/L in wine 1 (less fermentation time) and 1.15 and 0.570 g/L in wine 2 and wine 3, respectively. This fact can be contrary in other fermentation processes where tannins content increased due to the effect of their synthesis and/or extraction in an ethanol medium. Tannins in grape seeds are slowly dissolved during maceration as the cuticle is dissolved in ethanol; by contrast, tannins in grape skins were extracted more rapidly by the effect of their ready dissolution in the aqueous phase [30].

Table 1 shows the concentrations of flavonols and flavan-3-ol derivatives in blueberry juices and wines. Four flavonols were identified: two quercetin derivatives and two syringetin derivatives. Quercetin derivatives presented higher concentrations than those of syringetin in the juice from fresh fruit; in addition, the galactose derivatives were found in higher concentration in both cases. The drying process decreased the concentration of these phenolic compounds. Some authors found out that the drying process increases the concentration of flavonols [23]. During the fermentation process, a maceration process occurs with the berry skins, so these compounds should be extracted from skins; however, the concentration decreased, and this reduction can be ascribed to copigmentation reactions with anthocyanins [11]. The final concentration in wines is a balance between the extraction from skins and the reactions with other compounds, obtaining wine 2 with the highest flavonol concentration.

Also, five flavan-3-ol derivatives were identified and quantified: 3 monomers and 2 procyanidins. The major compound in juice from fresh fruit was procyanidin B1 followed by catechin. Drying and fermentation stages caused a decrease in concentration of total flavan-3-ol derivatives, although procyanidin B1 increased during drying and epicatechin could be quantified. These compounds are involved in different reactions. Catechins and proanthocyanidins are the main substrates for condensation with monomeric anthocyanins and their subsequent evolution to polymeric anthocyanins [31].

Figure 3 shows the values of antioxidant activity measured by DPPH assay in juices and wines from blueberries. As can be seen, the juice from fresh fruit had a high

Table 1: Flavan-3-ol and flavonols concentrations (mg/L) and homogenous groups for blueberry juices and wines at different fermentation times.

	Fresh juice	Juice after drying	Wine 1	Wine 2	Wine 3
Quercetin-3-O-galactoside	10.6 ± 0.862^{a}	4.93 ± 0.232^{b}	1.87 ± 0.104^{c}	2.18 ± 0.044^{c}	1.88 ± 0.011^{c}
Quercetin-3-O-glucoside	7.13 ± 1.03^{a}	4.04 ± 0.123^{b}	3.05 ± 0.221^{c}	3.68 ± 0.078^{bc}	3.08 ± 0.028^{c}
Syringetin-3-O-galactoside	4.25 ± 0.569^{a}	3.28 ± 0.249^{b}	2.06 ± 0.116^{d}	2.64 ± 0.084^{c}	2.61 ± 0.015^{c}
Syringetin-3-O-glucoside	2.08 ± 0.351^{a}	3.60 ± 0.292^{b}	3.10 ± 0.302^{c}	3.48 ± 0.039^{bc}	3.22 ± 0.160^{bc}
Total flavonols	24.1 ± 0.382^{a}	15.8 ± 0.897^{b}	10.1 ± 0.106^{c}	12.0 ± 0.247^{d}	10.8 ± 0.139^{c}
Procyanidin B1	19.5 ± 1.32^{a}	24.6 ± 1.68^{b}	14.9 ± 0.422^{c}	15.5 ± 0.120^{c}	17.0 ± 1.19^{a}
Epigallocatechin gallate	2.87 ± 0.064^{a}	2.78 ± 0.085^{a}	2.88 ± 0.336^{a}	3.12 ± 0.045^{a}	4.84 ± 0.650^{b}
Catechin	15.9 ± 1.19^{a}	5.38 ± 0.526^{b}	4.22 ± 0.776^{b}	4.95 ± 0.282^{b}	4.51 ± 0.557^{b}
Procyanidin B2	3.17 ± 0.622^{a}	2.80 ± 0.187^{a}	3.45 ± 0.673^{a}	3.37 ± 0.087^{a}	3.59 ± 0.609^{a}
Epicatechin	ND	2.20 ± 0.136^{a}	3.06 ± 0.134^{c}	2.26 ± 0.057^{a}	3.87 ± 0.542^{b}
Total flavan-3-ol	41.5 ± 0.687^{a}	37.8 ± 1.388^{b}	28.5 ± 1.321^{c}	29.2 ± 0.237^{c}	33.8 ± 0.117^{b}

ND: not detected. Values in the same row with different superscript letters are significantly different, $p = 0.05$.

antioxidant activity; this value decreased after the drying process. Wines 1 and 2 obtained from the sweet juice had no significant differences, whereas wine 3 (totally fermented wine) presented the lowest value. Antioxidant activity has been related to phenolic compound and vitamin C. Some authors have found out that anthocyanins are the main compounds responsible for the antioxidant activity of berry fruits, and vitamin C affects this activity less. Specifically, a linear relation between total anthocyanins and antioxidant activity has been found in blueberry varieties [32] and others have found that vitamin C did not contribute to the antioxidant activity values in blueberries [33]. Some authors state that the antioxidant activity is closely related to compounds of high molecular weight [34, 35]. In this work, the antioxidant activity decreased during the drying process and during the fermentation and there was no significant correlation between antioxidant activity, measured by the DPPH assay, and the composition of phenolic compounds, although there was a statistically significant relationship between antioxidant activity and vitamin C at the 99% confidence level, since the p value in the ANOVA is less than 0.01.

Figure 4 shows the scores obtained in the sensory analysis of the three wines. Wine 1 presented a more balanced score, standing out among others in terms of aroma. This can be due to the fact that the fermentation process was shorter, so varietal aromas were higher than in wine 2 and wine 3. Wine 2 also presented an acceptable score for the tasters, standing out among the others in terms of flavor, as a result of the compensation between the acid flavor of the blueberry and the sweetness of a semisweet wine. On the other hand, in spite of having the worst score in flavor and aroma, wine 3 presented the best score in color, as a consequence of the highest contact time with the solid parts during the fermentation.

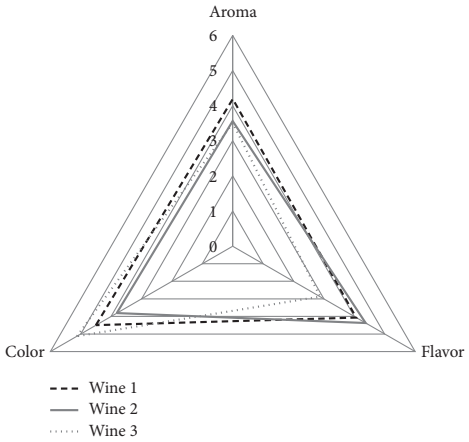

Figure 4: Sensory evaluation of the obtained wines.

4. Conclusions

The winemaking of blueberry wines caused changes in color and concentration in bioactive compounds. The drying process increased the absorbances at 420, 520, and 620 nm, so the browning index and the contribution of red compounds were higher in the juice after drying. The concentration of anthocyanins and tannins also increased with drying; however, flavonols, flavan-3-ol derivatives, and vitamin C decreased in this stage. The fermentation stage caused a decrease in phenolic compounds, tannins, and antioxidant activity, while the concentration of vitamin C was constant. Wine 3 presented lower values of anthocyanins and tannins as well as antioxidant activity, in addition to high volatile acidity. The sensory analysis showed that partially fermented wines had the best score. In this sense, in the production of blueberry wines, the drying process is suitable to obtain juices rich in sugars; partial fermentation obtains better results than total fermentation. Further study on the drying stage as well as the fermentation could improve the characteristics of these wines.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Licensed Content Author	Juan Martín-Gómez, M Ángeles Varo, Julieta Mérida, et al
Licensed Content Date	Mar 21, 2019
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Research Article

Received: 23 October 2018 Revised: 23 January 2019 Accepted article published: 22 February 2019 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9657

The influence of berry perforation on grape drying kinetics and total phenolic compounds

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Abstract

BACKGROUND: Drying is one of the traditional methods used for the conservation of fruits. In recent years, different methods have been developed to obtain higher quality products. Chamber-drying methods with hot air at controlled temperature are reliable and easy to use. The effect of piercing the structure of grape berries on their drying time was studied experimentally during convective drying within a temperature range of 30–50 °C. Experimental moisture loss results were fitted to different mathematical models, evaluated for goodness of fit by comparing their respective R^2, χ^2, and root mean square error.

RESULTS: The Midilli *et al.* model provided a better prediction to describe the drying of whole grapes than the other models evaluated. However, punched grapes showed a better fit for the two-term model at 30 and 40 °C, and the approximation of diffusion model at 50 °C. The values of effective moisture diffusivity fluctuated between 8.04×10^{-12} and 7.31×10^{-11} $m^2\ s^{-1}$. Activation energy was 56.49 and 54.43 kJ mol^{-1} for whole and punched grapes, respectively. All the drying processes produced an increase of total phenolic compounds and antioxidant activity in grapes, these increases being higher in whole grape drying.

CONCLUSION: The drying of punched grapes was faster and the activation energy higher than with drying of whole grapes; however, whole grapes presented more total phenolic compounds and antioxidant activity.

Keywords: grape; chamber-drying; kinetic models; total phenolic compounds; antioxidant activity

INTRODUCTION

Tempranillo is one of the most important grape varieties in Spain, being the main one of the Rioja Denomination of Origin. However, it has spread throughout the country; this variety is now authorized in 38 denominations of origin of Spain and is considered as the preferential variety in 14 of them.

Berry red fruits such as grapes are widely studied because of their composition of bioactive compounds;[1–3] they have antioxidant properties, and protect cells from damage caused by reactive oxygen species. Cell damage is caused by oxidative stress, due to an imbalance between antioxidants and reactive oxygen species, related to cancer, aging, atherosclerosis, ischemic injury, and inflammation as well as neurodegenerative diseases.[4–6] Drying is one of the traditional methods used for the conservation of fruits and vegetables.[7] It is safe and environmentally friendly, as it does not use chemical preservatives. The advantage of food dehydration is that it reduces the moisture content and the water activity value, which prevents the proliferation of microorganisms and degradation reactions of food.[8] Traditional sun drying, currently used in southern Spain (Region of Montilla-Moriles) and southern Italy for grape raisining, depends on the environmental conditions and is a process that can take a few days and even weeks or months to be carried out. However, it presents several disadvantages. In recent years, different methods have been developed to control conditions and obtain higher quality products. Chamber-drying methods with hot air at a controlled temperature are reliable and easy to use. They also avoid the disadvantages of traditional drying, such as time, losses caused by insect attack, climatic changes such as occasional rains or strong solar radiation, or the deterioration of products by attack from fungal toxins such as ochratoxin A.[9] Authors such as Martin-Gomez *et al.*[10] and Marquez *et al.*[11] have shown that the partial drying of berries is a good preservative method, as well as increasing the concentration of phenolic compounds and the antioxidant activity of the obtained fruits.

The aims of this work were to evaluate the improvement in drying performance by piercing the structure of the berry, developing predictive models of the kinetics of chamber-drying with hot air and observing the drying effect on the total phenolic compounds and antioxidant activity of the fruits.

MATERIALS AND METHODS

Materials

Grapes of Tempranillo variety (*Vitis vinifera* L.) were purchased by the Andalusian Institute for Research and Training in Agriculture, Fisheries, Food and Organic Production (IFAPA, Cabra, southern Spain). The grapes were harvested at the commercial optimum maturity stage, then labeled and stored at −18 °C. For thawing, the grapes were kept at 25 ° C for 24 h. The grapes were destemmed and divided into two batches. In the first, the destemmed grapes

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retained the pedicels (whole grapes); in the second batch the pedicels were removed from the destemmed grapes, so they had a perforation in the structure of the berry (punched grapes).

The initial moisture content of the grapes was obtained by the AOAC method, whereby the grapes were dried at 100 °C until constant weight. This value was 2.70 kg water kg^{-1} dry matter (d.m.).

Drying process

An amount of 3 kg of punched and whole grapes was dried in triplicate in a chamber at constant temperature and an initial relative humidity of 20% (Fig. 1). Both types of grapes (whole and punched grapes) were dried at 30, 40 and 50 °C, the relative humidity increased in the first 2 h of drying to 42% and then decreased to 20% to the end of the process. All the drying processes were carried out for 72 h, performing a periodical weight loss control.

Mathematical models

The data obtained from the drying processes were adjusted to different mathematical models frequently used to model drying curves. For this, the moisture ratio (MR) was calculated using the following formula:

$$\mathrm{MR} = \frac{M_t - M_e}{M_0 - M_e} \tag{1}$$

where M_t, M_0 and M_e represent the moisture content at any time of drying (kg water kg^{-1} d.m.), initial moisture content (kg water kg^{-1} d.m.) and equilibrium moisture content (kg water kg^{-1} d.m.), respectively. The moisture content at equilibrium is small relative to the others; it can be assumed that Eqn (1) can be simplified as follows:[12–15]

$$\mathrm{MR} = \frac{M_t}{M_0} \tag{2}$$

The data were analyzed using Statgraphics Centurion XVI.I software, where the model constants and coefficients were determined by nonlinear regression, based on the Marquardt algorithm. To evaluate the adjustment of the mathematical models with regard to the experimental data, the coefficient of determination (R^2), the reduced chi-squared (χ^2) and root mean square error (RMSE) were used; a high value of R^2 and a low value of χ^2 and RMSE established a better fit. These parameters were calculated from the following equation:[7]

$$R^2 = 1 - \frac{\sum_{i=1}^{N} \left(\mathrm{MR}_{\mathrm{exp},i} - \mathrm{MR}_{\mathrm{pre},i}\right)^2}{\sum_{i=1}^{N} \left(\overline{\mathrm{MR}}_{\mathrm{exp}} - \mathrm{MR}_{\mathrm{exp},i}\right)^2} \tag{3}$$

$$\chi^2 = \frac{\sum_{i=1}^{N} \left(\mathrm{MR}_{\mathrm{exp},i} - \mathrm{MR}_{\mathrm{pre},i}\right)^2}{n - z} \tag{4}$$

$$\mathrm{RMSE} = \left[\frac{1}{N} \sum_{i=1}^{N} \left(\mathrm{MR}_{\mathrm{pre},i} - \mathrm{MR}_{\mathrm{exp},i}\right)^2\right]^{\frac{1}{2}} \tag{5}$$

where $\mathrm{MR}_{\mathrm{exp},i}$ and $\mathrm{MR}_{\mathrm{pre},i}$ are the experimental and predicted moisture ratio respectively, N is the number of observations and z are the constant numbers.

Determination of effective diffusivity

The effective diffusivity was calculated for each drying process, using the analytical solution of Fick's second law for spherical geometry assuming that the shape of the grape is spherical, the shrinkage of the fruit negligible, the migration of humidity is due to diffusion and constant coefficients and temperature:[7,16–19]

$$\mathrm{MR} = \frac{6}{ß^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{n^2 \pi^2 D_{\mathrm{eff}}\, t}{R^2}\right) \tag{6}$$

where D_{eff} is the effective diffusivity ($m^2\ s^{-1}$), R is the equivalent radius of the grape (m) and t is time (s). When the drying process is long the equation can be simplified:

$$\ln\ (\mathrm{MR}) = \ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_{\mathrm{eff}}}{R^2}\right) t \tag{7}$$

In order to calculate the effective diffusivity coefficient from the previous equation, it is necessary to represent the natural logarithm of the moisture relation *versus* time. The slope of the line they form is given by

$$\mathrm{Slope} = \frac{D_{\mathrm{eff}}\, \pi^2}{R^2} \tag{8}$$

Calculation of activation energy

The effective diffusivity depends on the temperature and is calculated from the Arrhenius equation:[20]

$$D_{eff} = D_0\ \exp\left(-\frac{E_a}{R\ (T + 273.15)}\right) \tag{9}$$

where D_0 is the pre-exponential factor of the Arrhenius equation ($m^2\ s^{-1}$), E_a is the activation energy (kJ mol^{-1}), R is the universal gas constant (kJ $mol^{-1}\ K^{-1}$) and T is air temperature (°C).

The activation energy is calculated from the slope of the line of the natural logarithm of the effective diffusivity coefficient *versus* the inverse of the temperature:

$$\ln\ (D\mathrm{eff}) = \ln\ (D0) - \frac{E_a}{R\ (T + 273.15)} \tag{10}$$

$$\mathrm{Slope} = -\frac{E_a}{R} \tag{11}$$

Extraction process

0.2 g of fresh grapes or 0.1 g of dried grapes were treated with 3 mL of acidified methanol (0.1% HCl), introducing the mixture in ultrasound for 10 min. The supernatant was removed and 3 mL acidified methanol was added; the procedure was repeated three times. All the extract was centrifuged for 10 min at 4000 rpm and the supernatant was made up to 10 mL. The extract was filtered through a 0.45 µm Nylon filter before analysis.

Total phenolic compounds (TPC)

TPC content was estimated in triplicate using the Folin–Ciocalteu method, with some modifications. 1.250 mL Folin–Ciocalteu reagent, diluted 1:5 in distilled water, was added to 50 µL of extract filtered through a 0.45 µm filter. After vigorous agitation and incubation for 1 min at room temperature (25 °C), 1 mL of 10% sodium carbonate was added. The mixture was again stirred and allowed

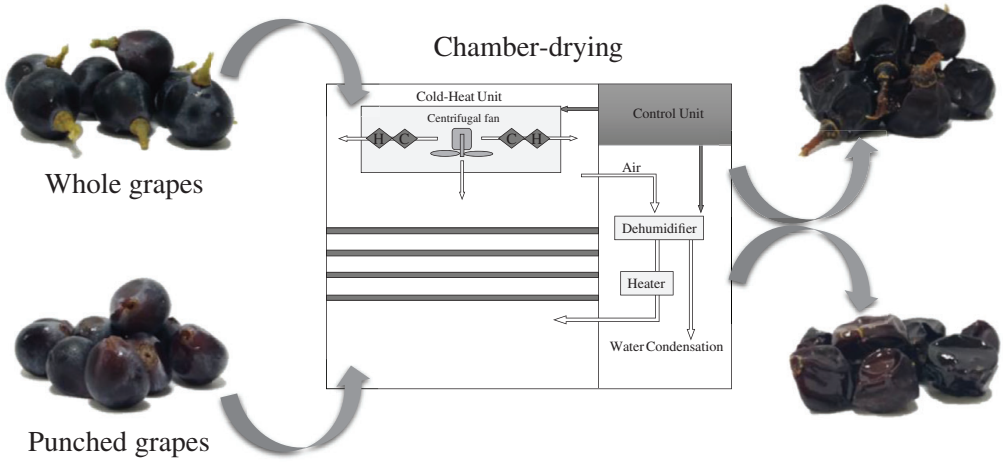

Figure 1. Scheme of the chamber- drying and changes produced in the grapes.

to react for 30 min in darkness at room temperature. After this time, the absorbance at 760 nm was measured using a UV–visible spectrophotometer (Beckman DU 640). A gallic acid calibration curve was performed using different concentrations of gallic acid standard (1, 0.75, 0.5, 0.25, 0.1, 0.05 and 0.01 mg gallic acid mL^{-1}). Total phenolic content was expressed in milligrams of gallic acid per gram of fruit.

Antioxidant activity

Antioxidant activity was analyzed in triplicate through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Katalinic *et al.*,[21] with some modifications. A 45 mg L^{-1} solution of DPPH in methanol was prepared on a daily basis and stored in darkness. The analytical procedure was as follows: a 200 µL aliquot of extract filtered through 0.45 µm was placed in a cell and 3 mL of a 45 mg L^{-1} solution of DPPH in methanol was then added. A control sample (200 µL of water + 3 mL DPPH solution) was also prepared in parallel. Following vigorous stirring, the absorbances at 517 nm of the control sample was measured in a Beckman DU 640 spectrophotometer. The sample was measured under identical conditions after 30 min of incubation at room temperature. A Trolox calibration curve was performed using concentrations between 10 and 200 mg Trolox L^{-1}. The results were expressed as Trolox equivalents (mg Trolox L^{-1}) using the following equation to calculate the inhibition percentage:

$$\%\text{inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \qquad (12)$$

RESULTS AND DISCUSSION

Drying characteristic of Tempranillo grape

The drying process was represented by the moisture ratio, determined as the ratio of the moisture content at each time interval to the initial moisture content, *versus* the drying time (Fig. 2). As can be seen, the moisture ratio decreased exponentially over time.

Figure 2(A) shows the temperature effect on the drying time in the whole grapes or grapes with the pedicel. As can be seen in the figure, the higher temperature produced a greater decrease in the humidity ratio. The moisture ratio value was 0.538 at 30 °C at 72 h of drying, and comparing this value in the other processes, the process was found to be reduced by 46% and 70% at 40 and 50 °C, respectively. This fact implied that the exposure of the berry to a higher temperature favored the process of water elimination. Figure 2(B) shows the changes in moisture ratio of the punched grapes at the same temperatures. In this case the value of moisture ratio at 30 °C at 72 h of drying was 0.214. This value at 40 °C was reached at a time 26% lower, and at 50 °C at a time 68% lower. The temperature increased the water evaporation rate of the fruit.

Figure 2 shows the effect of the fruit damage in the drying process kinetics. Comparing the drying processes at 30 °C, the whole grapes needed 72 h to reach a moisture ratio of 0.538, and the punched grapes reached this value in 36 h; the process was achieved in half the time. In the processes at 40 °C, the whole grapes reached a moisture ratio of 0.281 at the end of the drying, this value being reached in 45 h by the punched grapes (37.5% less). Finally, at the temperature of 50 °C, the punched grapes took 47% less time to reach the minimum moisture ratio value obtained by whole grapes. The data obtained indicate that perforation of the berry favors water evaporation, minimizing the resistance offered by the wax of the grape skin to water evaporation.[22] Authors such as Serratosa *et al.*[23] reduced the drying time by 24%, using a pre-treatment by dipping the fruit in alkaline solutions to reduce the skin's resistance, while the loss of integrity of the berry can produce even a 50% decrease in the drying time.

Mathematical modeling of drying curves

The experimental values of the moisture ratio obtained from the drying processes at different temperatures were adjusted to seven mathematical models[24–30] (Table 1). The values of the statistical parameters are shown in Tables 2 and 3. In all cases, the correlation coefficient for the models was above 0.97, indicating a good fit. The R^2, χ^2 and RMSE values were between 0.9762 and 0.9985, 0.000043 and 0.007671, 0.008477 and 0.085120, respectively. The simplicity of the model could be used to select the best fit when the statistical parameters are very closed; however, the best fit was established for the model with the highest value of R^2 and lowest value of χ^2 and RMSE, which was used as the optimal

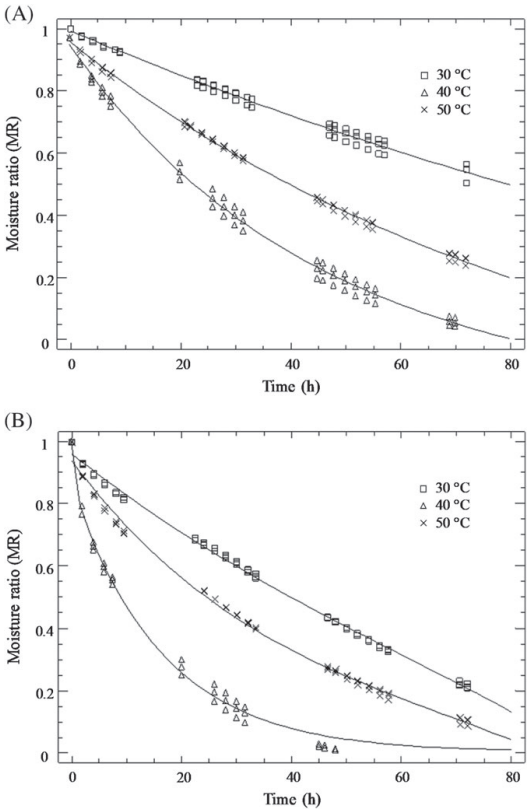

Figure 2. Changes in moisture ratio during the controlled dehydration processes, of (A) whole grapes at 30, 40 and 50 °C, and (B) punched grapes at 30, 40 and 50 °C.

criterion to evaluate the quality of the adjustment to the proposed models. Therefore, based on the values of R^2, χ^2 and RMSE, it was shown that, of the models evaluated, the Midilli *et al.* model had a better fit and allowed a better description of the drying characteristics for the whole grape at all the temperatures studied. However, in the case of punched grapes the Two-term model best described the drying characteristics at temperatures of 30 and 40 °C, while the Approximation of diffusion model presented the best adjustment to the temperature of 50 °C.

Determination of the effective diffusivity and activation energy

Values of the effective diffusivity, calculated from the slope of the line obtained by the natural logarithm of the moisture ratio *versus* time, are shown in Table 4 with a range of values between 8.04 × 10^{-11} and 7.31 × 10^{-11} m^2 s^{-1}. As can be seen, D_{eff} increased with the increment of air temperature. In addition, damage to the berries also produced a slight increase of the values of this parameter. Drying at 50 °C of the punched grapes had the highest value of D_{eff} and therefore the highest water diffusion and evaporation, the drying of whole grapes at 30 °C being those that presented a lower water diffusion. The D_{eff} values obtained were collected within the general desirable range for the drying of food materials, between 10^{-12} and 10^{-8} m^2 s^{-1}.[31]

Table 1. Selected drying models for describing grapes drying processes

Model name	Model	Reference
Newton	$MR = \exp(-kt)$	24
Henderson and Pabis	$MR = a\exp(-kt)$	25
Logarithmic	$MR = a\exp(-kt) + c$	26
Wang and Singh	$MR = 1 + at + bt^2$	27
Two-term	$MR = a\exp(-k_0 t) + b\exp(-k_1 t)$	28
Approximation of diffusion	$MR = a\exp(-kt) + (1-a)\exp(-kbt)$	29
Midilli *et al.*	$MR = a\exp(-kt) + bt$	30

The activation energy is defined as the energy needed to remove 1 mol of water from the material to be dried, and is calculated through the slope of the line formed by plotting the natural logarithm of the effective diffusivity *versus* the inverse of the temperature. The values obtained from this parameter were 56.49 and 54.43 KJ mol^{-1} (Table 4) for whole and punched grapes, respectively. Since in both cases the studied sample was grape, these data showed similar values, even though the corresponding slight difference could be due to the fact that the grapes that retained their pedicels needed a greater amount of energy to evaporate the same amount of water from the fruit.

Total phenolic content and antioxidant activity

TPC was measured by the Folin–Ciocalteu method in all the drying processes carried out (Fig. 3A). As can be seen, values of TPC increased with the increment of temperature because these processes are influenced by the concentration effect due to water evaporation. Comparing the treatment of the grapes, the whole grapes presented higher values than punched grapes, except at 50 °C, where the values were similar. The TPC increased 1.86, 2.5 and 2.88 times the initial value for 30, 40 and 50 °C, respectively. In the drying of the punched grapes, the increases were 1.48, 1.67 and 2.82 times the initial value for 30, 40 and 50 °C, respectively – lower than in the whole grapes. Considering that the moisture content was lower in the punched grapes, the concentration effect should be higher in these grapes and the TPC values should be higher; therefore, the composition of damaged grapes (without pedicel) was more affected by the temperature. The increase in TPC during the drying process of fruit has been reported by authors such as Zhao *et al.*[32] They demonstrated that TPC increased during the drying of wolfberries at 40, 50 and 60 °C. However, other authors have reported a decrease of these compounds during the drying of berries such as strawberry.[33] Some mechanisms can produce a decrease in phenolic compounds; for example, release of bound phenolic compound, partial degradation of lignin or thermal degradation.[33] Authors such as Henríquez *et al.*[34] concluded that the degradation reactions of phenolic compounds increase with the drying temperature, and these compounds could take part in enzymatic and non-enzymatic reactions, the formation of insoluble oxidation compounds, polymer production and thermolabile compound degradation.[35] The final content of total phenolic compounds is a balance between the concentration effect due to water evaporation and the mechanisms to reduce their concentration.

Table 2. Statistical analysis at different temperatures for whole grape drying

Model	Temperature (°C)	χ^2	RMSE	R^2
Newton	30	0.000209	0.014353	0.9887
	40	0.000238	0.015326	0.9952
	50	0.000756	0.027178	0.9914
Henderson and Pabis	30	0.000211	0.014302	0.9887
	40	0.000239	0.015233	0.9953
	50	0.000765	0.027177	0.9914
Logarithmic	30	0.000242	0.015169	0.9873
	40	0.000097	0.009651	0.9981
	50	0.000431	0.020202	0.9952
Wang and Singh	30	0.000205	0.014088	0.9891
	40	0.000113	0.010492	0.9977
	50	0.000759	0.027255	0.9914
Two-term	30	0.000196	0.013562	0.9899
	40	0.000079	0.008641	0.9985
	50	0.000439	0.020212	0.9952
Approximation of diffusion	30	0.000209	0.014134	0.9890
	40	0.000155	0.012194	0.9969
	50	0.000724	0.026205	0.9920
Midilli *et al.*	30	0.000167	0.012935	0.9902
	40	0.000075	0.008477	0.9985
	50	0.000043	0.020183	0.9953

Table 3. Statistical analysis at different temperatures for punched grape drying

Model	Temperature (°C)	χ^2	RMSE	R^2
Newton	30	0.000803	0.028121	0.9851
	40	0.001013	0.031574	0.9861
	50	0.002352	0.047820	0.9767
Henderson and Pabis	30	0.000778	0.027471	0.9858
	40	0.000579	0.023694	0.9922
	50	0.001422	0.036645	0.9863
Logarithmic	30	0.000296	0.01681	0.9947
	40	0.000415	0.019885	0.9945
	50	0.001462	0.036611	0.9863
Wang and Singh	30	0.000548	0.023059	0.9900
	40	0.001765	0.041349	0.9762
	50	0.007671	0.085120	0.9767
Two-term	30	0.000176	0.012864	0.9969
	40	0.000386	0.019016	0.9950
	50	0.000646	0.023969	0.9941
Approximation of diffusion	30	0.000750	0.026755	0.9865
	40	0.000432	0.020295	0.9943
	50	0.000554	0.022530	0.9948
Midilli *et al.*	30	0.000182	0.013209	0.9967
	40	0.000404	0.019612	0.9946
	50	0.001462	0.036611	0.9863

Figure 3(B) shows the antioxidant activity values, measured as the inhibition percentage of the DPPH radical. The values increased during the drying processes, this increase being higher in the whole grapes than in the punched grapes. In whole grapes, the antioxidant activity increased 2.77 times at 30 °C and 3.1 times at 40 and 50 °C. In the drying of punched grapes, the increases were 1.6, 1.9 and 2.7 times at 30, 40 and 50 °C, respectively. Antioxidant activity was related to phenolic compounds. The content of these compounds reached higher values in the drying of whole grapes, so the antioxidant activity was also higher, although regression analysis did not show a significant correlation between either value, due to other compounds that could affect the antioxidant activity.

CONCLUSIONS

The influence of temperature on grape drying kinetics showed a reduction of time with increase of temperature, this reduction

Table 4. Activation energy and effective diffusivity of whole and punched grapes

Sample	Activation energy (kJ mol^{-1})	Temperature (°C)	Effective diffusivity (m^2 s^{-1})
Whole grape	56.49 ± 6.29	30	$8.04 \times 10^{-12} \pm 6.71 \times 10^{-13}$
		40	$1.66 \times 10^{-11} \pm 5.83 \times 10^{-13}$
		50	$3.22 \times 10^{-11} \pm 2.39 \times 10^{-12}$
Punched grape	54.43 ± 4.00	30	$1.90 \times 10^{-11} \pm 3.27 \times 10^{-13}$
		40	$2.86 \times 10^{-11} \pm 1.26 \times 10^{-12}$
		50	$7.31 \times 10^{-11} \pm 6.33 \times 10^{-12}$

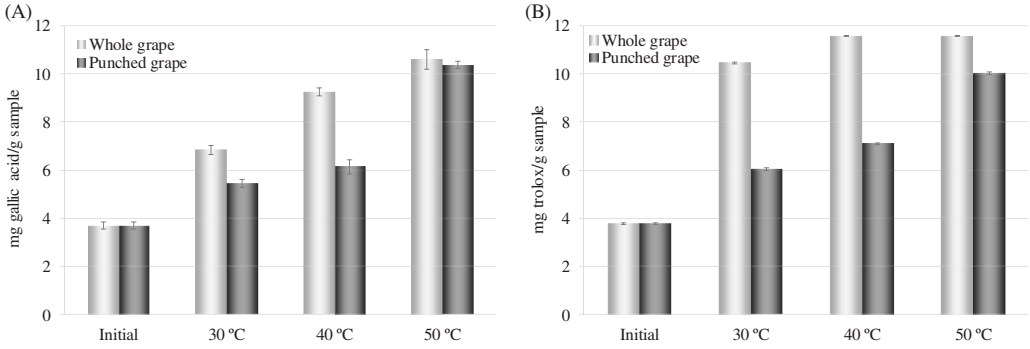

Figure 3. (A) Total phenolic content and (B) antioxidant activity in drying processes of the whole and punched grapes.

being up to 70% at 50 °C in respect to 30 °C. Grape damage favored the process of water evaporation, decreasing the drying time by 50% in respect to whole grapes.

The Midilli *et al.* model showed the best fit to describe the drying of whole grapes. However, the punched grapes had a better fit with the Two-term model at 30 and 40 °C, and the Approximation of diffusion model at 50 °C.

The effective diffusivity coefficient increased with temperature and perforation of the grape; values obtained were in the range from 8.04×10^{-12} to 7.31×10^{-11} m^2 s^{-1}, and values of the activation energy showed that less energy was needed to eliminate the water of the punched grapes (54.43 KJ mol^{-1}) than of the whole grapes (56.49 KJ mol^{-1}).

In relation to the content of total polyphenols and the antioxidant activity, an increase was observed in all the drying processes compared to the initial content, due to the concentration effect by the loss of water. But it was in the drying processes of whole grapes where the phenolic compounds were more stable in respect to the temperature and more content was obtained.

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Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (Vaccinium corymbosum)

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Publication: LWT - Food Science and Technology

Publisher: Elsevier

Date: Available online 6 December 2019

LWT - Food Science and Technology 120 (2020) 108931

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Influence of drying processes on anthocyanin profiles, total phenolic compounds and antioxidant activities of blueberry (*Vaccinium corymbosum*)

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ARTICLE INFO

Keywords:
Blueberry
Kinetic drying
Phenolic compounds
Anthocyanins
Antioxidant activity

ABSTRACT

This work studies blueberry convective drying-process at 30, 40 and 50 °C, with the aim to obtain juices with the highest concentration of phenolics compounds and antioxidant activity. To predict changes in moisture content versus drying time, the results were adjusted to 5 mathematical models to evaluate the best fit, which is the one with the highest value of the coefficient of determination (R^2) and lowest values the reduced chi square (χ^2) and the root mean square error (RMSE). The *Page* model was the best model for the drying processes at 30 and 50 °C, while at 40 °C, the best model was the *Approximation of diffusion* model. The values of effective moisture diffusivity ranged between 2.58×10^{-11} and 4.58×10^{-11} m^2/s, and the activation energy was 23.13 kJ/mol. The increase in temperature increased the absorbances at 420, 520 and 620 nm of the juice, the total phenolic contents (TPC) values and the anthocyanin concentration. The antioxidant activity, evaluated by the DPPH and ABTS methods, increased with the drying temperature. The best TPC values, antioxidant activity, anthocyanins concentration and color parameters were obtained from blueberries dried at 50 °C, being the most effective process in removing water.

1. Introduction

The most cultivated blueberries worldwide belong to the genus *Vaccinium* and species *corymbosum* and are native to North America and regions of Europe (Reque et al., 2014). These blueberries are of high quality and comprise 85% of the world production of the fruit (Garcia, Garcia, & Ciordia, 2013, pp. 5–8; Miyashita, Inoue, Yamada, & Ogiwara, 2018). The American continent is the largest producer of blueberries, accumulating three-quarters of the world production (Hidalgo & Almajano, 2017). Blueberries are small berry fruits that are sweet and juicy with an intense blue color and high content of phenolic compounds, such as phenolic acids, tannins, stilbenes, lignans and flavonoids, including anthocyanins, flavonols and flavanols (Guiné, Matos, Gonçalves, Costa, & Mendes, 2018; Nile & Park, 2014).

Phenolic compounds are known to have beneficial health properties; anthocyanins, the pigments responsible for the color of the berries, can represent up to 70% of the phenolic compound contents of blueberries (Olas, 2018). They are antioxidant compounds that can react with reactive oxygen species (ROS) to reduce the effects of oxidative stress, which gives them anti-inflammatory, cardioprotective, anticancer, neuroprotective, anti-aging, oculo-protective, and renal-protective properties. They also function against type 2 diabetes and have other dietary properties, reducing the risk of obesity (Das & Mandal, 2018; Fairlie-Jones, Davison, Fromentin, & Hill, 2017; Lee et al., 2017; Leong, Show, Lim, Ooi, & Ling, 2018; Pavlidou, Giaginis, Fasoulas, & Petridis, 2018; Różańska & Regulska-Ilow, 2018; Singh, Bishayee, & Pandey, 2018).

Drying is one of the oldest food preservation methods, since reducing the moisture content of food prevents the proliferation of microorganisms (Vega-Gálvez, Lemus-Mondaca, Tello-Ireland, Miranda, & Yagnam, 2009). In this process, the application of energy is necessary to achieve a change of state, sublimation or evaporation inside the food, resulting in water loss (Nemzer, Vargas, Xia, Sintara, & Feng, 2018). Different types of drying can be applied according to the method of heat transfer used, such as drying by conduction, convection or radiation (Kudra & Mujumdar, 2009).

From an oenological point of view, drying is a very important process in different parts of the world, such as southern Spain or southern Italy. Since many wines are made from dried grapes, radiation drying has traditionally been the most widely used, where the sun is the carrier of thermal energy; this process is known as “soleo”. This type of drying has many disadvantages, such as losses due to inclement weather (rain, hail etc.), variable temperatures at different times of day, and losses due to insect attacks, among others (Marquez, Perez-Serratosa, Varo, & Merida, 2014; Marquez, Serratosa, Lopez-Toledano, & Merida, 2012; Serratosa, Lopez-Toledano, Medina, & Merida, 2008).

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https://doi.org/10.1016/j.lwt.2019.108931
Received 24 May 2019; Received in revised form 4 December 2019; Accepted 5 December 2019
Available online 06 December 2019

There are different types of controlled drying, such as convection drying in a chamber, where a constant temperature and humidity value can be adjusted to desired levels, and the disadvantages discussed above are minimized. Authors such as Serratosa et al. (Serratosa et al., 2008) showed that in the drying of grapes, in which drying by "soleo" is still used, chamber-drying resulted in products with reduced loss of bioactive compounds, in addition to shortening the exposure time, reducing costs and increasing the quality of the product. The kinetic study of food drying processes is based on the study of the temporal evolution of moisture content in foods. For this, many authors (Henderson & Pabis, 1961; Page, 1949; Wang & Singh, 1978) have defined kinetic models that allow predicting the behavior of foods during drying process.

One of the disadvantages of winemaking of the blueberries is the low sugar content that they present, for this reason after the fermentation would be obtained drinks with an alcoholic content of around 5–6% (Johnson, de Mejia, Fan, Lila, & Yousef, 2013; Su & Chien, 2007), to avoid this inconvenience the sugar content of the fruit may be concentrated after partial dehydration of the berry (Martin-Gomez, Varo, Merida, & Serratosa, 2017). In addition, the fruit drying processes cause the loss of phenolic compounds and antioxidant activity due to the degradation reactions of these compounds that can be favored by the temperature (Lohachoompol, Srzednicki, & Craske, 2004; Martin-Gomez et al., 2017). Therefore, the aim of this work was to obtain enriched juices from dried blueberries, with high values of phenolic compounds and antioxidant activity, for this, the influence of temperature on the kinetics of the drying process and on the juice composition was evaluated.

2. Materials and methods

2.1. Chemicals

Folin Ciocalteu reagent, sodium carbonate, acid formic, acetonitrile, potassium peroxodisulfate, ethanol and methanol were obtained from Merck (Madrid, Spain). Anthocyanins were purchased by Extrasynthese (Genay, France). Gallic acid, DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and Trolox were from Sigma-Aldrich Chemical Co. (Madrid, Spain). All solvents used in this investigation were high-performance liquid chromatography (HPLC) grade.

2.2. Materials

Blueberries of the Ventura variety (*Vaccinium corymbosum*) were purchased by a Company from Huelva (southern Spain). The blueberries were harvested at the commercial optimum maturity stage and stored at −18 °C. For thawing, the blueberries were kept at 25 °C for 24 h.

The initial moisture content of the blueberries was obtained by the AOAC method (Intl, 2016). The blueberries were then dried at 100 °C until a constant weight was recorded. This value was 5.87 kg of water/kg of dry matter (kg water/kg d.m.).

2.3. Drying process

The blueberries were dried with an initial relative humidity of 20% and a constant air temperature of 30, 40 and 50 °C in a Frisol Climatronic chamber. Each drying process at different temperatures was performed in triplicate. All the drying processes were carried out by performing periodical weight loss measurements (every 2 h, during daylight hours) until the berries reached 50% of their initial moisture content, that was, when its moisture content was 2.94 kg water/kg d.m. A laboratory balance with accuracy at 0.02 g was used to achieve these measurements.

The fresh blueberries (after thawing) were manually pressed, obtained the juice (J0), and blueberries dried at 30, 40 and 50 °C were also pressed manually, to obtain their juices, (J30, J40 and J50 respectively). All juices were centrifuged at 2800 g, filtered with Millipore (Billerica, MA) HA filters of 0.45 μm pore size, and analyzed in triplicate.

2.4. Mathematical models

The data obtained from the drying processes were adjusted to different mathematical models frequently used to model drying curves. For this, the moisture ratio (MR) was calculated using the following equation:

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (1)$$

where Mt, M_0 and Me are the moisture content at a given drying time (kg water/kg dm), the initial moisture content (kg water/kg dm) and the equilibrium moisture content (kg water/kg dm), respectively. As the equilibrium moisture content is small relative to the others, it can be assumed that it is equal to zero (I. Doymaz, Tugrul, & Pala, 2006), simplifying the equation to:

$$MR = \frac{M_t}{M_0} \quad (2)$$

The data were analyzed with Statgraphics Centurion XVI. I software, where the model constants and coefficients were determined by non-linear regression, based on the Marquardt algorithm. To evaluate the fit of the mathematical models and the experimental data, the coefficient of determination (R^2), the reduced chi square (χ^2) and the root mean square error (RMSE) were used, a high value of R^2 and low values of χ^2 and RMSE denote a better fit. These parameters were calculated with the following equations (İ. Doymaz & İsmail, 2011):

$$R^2 = 1 - \frac{\sum_{i=1}^{N} (MR_{exp,i} - MR_{pre,i})^2}{\sum_{i=1}^{N} (\overline{MR}_{exp} - MR_{exp,i})^2} \quad (3)$$

$$\chi 2 = \frac{\sum_{i=1}^{N} (MR_{exp,i} - MR_{pre,i})^2}{n - z} \quad (4)$$

$$RMSE = \left[\frac{1}{N} \sum_{i=1}^{N} (MR_{pre,i} - MR_{exp,i})^2 \right]^{\frac{1}{2}} \quad (5)$$

where $MR_{exp,i}$ and $MR_{pre,i}$ are the experimental and predicted moisture ratios, respectively, N is the number of observations and z is a constant.

2.5. Determination of effective diffusivity

The effective diffusivity was calculated for each drying process using the analytical solution of Fick's second law for spherical geometry, assuming that blueberry shape is spherical, the shrinkage of the fruit is negligible, and the migration of humidity is due to diffusion with a constant coefficient and temperature.

$$MR = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} exp\left(-\frac{n^2 \pi^2 D_{eff}\, t}{R^2} \right) \quad (6)$$

where D_{eff} is the effective diffusivity (m^2/s), R is the equivalent radius of blueberries (m) and t is time (s). When the drying process is long, the equation can be simplified.

$$Ln\,(MR) = Ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{R^2}\right) t \quad (7)$$

To calculate the effective diffusivity coefficient from the previous equation, it is necessary to represent the natural logarithm of the moisture relation versus time. The slope of the line they form is given by:

$$Slope = \frac{D_{eff}\,\pi^2}{R^2} \quad (8)$$

2.6. Determination of activation energy

The effective diffusivity depends on the temperature and is calculated from the Arrhenius equation (Simal, Femenia, Cárcel, & Rosselló, 2005):

$$D_{eff} = D_0 \exp\left(-\frac{E_a}{R\,(T+273.15)}\right) \quad (9)$$

where D_0 is the pre-exponential factor of the Arrhenius equation (m^2/s), E_a is the activation energy (kJ/mol), R is the universal gas constant (kJ/mol K) and T is the air temperature (°C).

The activation energy is calculated from the slope of the line of the natural logarithm of the effective diffusivity coefficient versus the inverse of the temperature.

$$Ln\,(Deff) = Ln\,(D0) - \frac{E_a}{R\,(T+273.15)} \quad (10)$$

$$Slope = -\frac{E_a}{R} \quad (11)$$

2.7. Spectrophotometric determinations

The optical density of the juices from undried (J0) and dried blueberries at 30 °C (J30), 40 °C (J40) and 50 °C (J50) were measured on a UV–vis spectrophotometer (Beckman DU 640) using quartz cells with a 1 mm light path, which were corrected for a path length of 1 cm. Absorbance was read at 420, 520 and 620 nm, which represented the contribution of brown, red and blue colors, respectively (Martin-Gomez et al., 2017). The hue indicates the relation between the absorbance measured at 420 and 520 nm.

2.8. Total phenolics contents (TPC)

The total phenolic compound values of the juices were estimated using the Folin-Ciocalteu method (Varo et al., 2018). The absorbance at 760 nm was measured using a UV–vis spectrophotometer (Beckman DU 640). A gallic acid calibration curve was performed using different concentrations of gallic acid standard (1, 0.75, 0.5, 0.25, 0.1, 0.05 and 0.01 g gallic acid/L). The total phenolic content was expressed in g gallic acid equivalents/L (g GAE/L).

2.9. Antioxidant activity

2.9.1. DPPH method

The antioxidant activity was analyzed using the DPPH assay according to Katalinic et al. (Katalinic, Milos, Kulisic, & Jukic, 2006) with some modifications. A 45 mg/L solution of DPPH in methanol was prepared daily and stored in darkness. The analytical procedure was as follows: a 200 μL aliquot of sample diluted at 1:10 was placed in a cell, and 3 mL of a 45 mg/L solution of DPPH radical in methanol was then added. A control sample (200 μL of water + 3 mL of DPPH radical solution) was also prepared in parallel. Following vigorous stirring, the absorbances of the control sample at 517 nm were measured in a Beckman DU 640 spectrophotometer. The samples were measured under identical conditions after 30 min of incubation at room temperature. The inhibition percentage was calculated using equation (5). A Trolox calibration curve was prepared and the results were expressed as Trolox equivalents (g Trolox/L).

$$\text{Antiradical effect} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \cdot 100 \quad (12)$$

2.9.2. ABTS method

Total antioxidant activity was measured by discoloration of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation according to the method described by Re et al. (Re et al., 1999). Where $ABTS^{\cdot+}$ was produced by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate, this mixture was kept in the dark at room temperature for 12–16 h before use. The resulting $ABTS^{\cdot+}$ solution was diluted in a 20 mM phosphate buffer at pH 7.4 until the absorbance at 734 nm was 0.700 ± 0.020, as measured in the Beckman DU 640 spectrophotometer. For the analysis of the antioxidant activity, 900 μL of the $ABTS^{\cdot+}$ solution dilution was mixed with 100 μL of the sample diluted to 1:100. After 6 min, the absorbance at 734 nm was measured next to a blank with distilled water. The inhibition percentage was calculated using equation (6). A Trolox calibration curve was prepared and the results were expressed as Trolox equivalents (g Trolox/L).

$$\text{Antiradical effect} = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \cdot 100 \quad (13)$$

2.10. Identification and quantification of anthocyanins by HPLC-DAD

A 50 μL sample of the filtered juice was injected into an HPLC-DAD instrument (Beckman Coulter System Gold, 168 Detector), and the analyses were carried out on a LiChrospher 100 RP-18 column (250 mm × 4.6 mm, 5 μm), using 10% aqueous formic acid in HPLC-grade water (solvent A) and 10% formic acid, 45% acetonitrile and 45% HPLC-grade water (solvent B), as a mobile phase, at a flow rate of 1 mL/min. The absorbance at 520 nm was used to quantify anthocyanin, according to the method proposed by Marquez et al. (Marquez et al., 2012). The identification was carried out by comparing the retention times of the samples with those of the standards and recording their UV–Vis spectra. For the quantitative analysis of anthocyanins, a calibration curve was obtained by injection of different concentration of malvidin 3-*O*-galactoside. All the anthocyanins were quantified as malvidin 3-*O*-galactoside, which is the main anthocyanin in the studied blueberry.

2.11. Statistical analysis

The results for all samples were subjected to analysis of variance at the 99.0% confidence level, in addition homogeneous groups were calculated in order to stablish significates differences between means. A simple linear correlation has been made between antioxidant activity values and total phenolic content TPC. The software used was the Statgraphics Centurion XVI.

3. Results and discussion

3.1. Drying characteristic of blueberry ventura

The drying processes can be followed through the drying curves, where the change in the moisture relation (MR) versus time is represented. Fig. 1 shows the drying curves of the blueberries at different temperatures. An increase in temperature produced a greater rate in the process of removing water from the berries. The drying process was stopped when the moisture content was reduced to 50% of the initial value, a humidity ratio of 0.5. At 50 °C, this value was reached in 14 h, this time increased to 24 h at 40 °C and to 32 h at 30 °C, so the drying process performed at 40 °C reduced the time by 25% compared to drying at 30 °C, and drying at 50 °C reduced the time 56% compared to drying at 30 °C and 42% compared to drying at 40 °C.

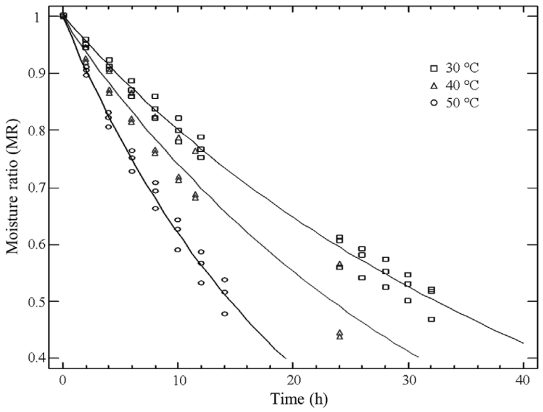

Fig. 1. Drying curves of blueberries dried at 30, 40 and 50 °C.

3.2. Mathematical modeling

The data obtained from the blueberry drying processes at different temperatures were adjusted to 5 mathematical models, as shown in Table 1 (Henderson & Pabis, 1961; Madamba, Driscoll, & Buckle, 1996; Page, 1949; Wang & Singh, 1978; Yaldiz, Ertekin, & Uzun, 2001). The optimal criteria to evaluate the best fit and the quality of the adjustment to the proposed models was the highest value of R^2 and lowest values of χ^2 and RMSE. The data obtained for these parameters are shown in Table 2. The R^2 values were between 0.9879 and 0.9908, the $\chi 2$ values were between 0.00030 and 0.00036 and the RMSE values were between 0.01672 and 0.01758, indicating that a good fit was obtained for all the models proposed in Table 1. More specifically, the *Page* model presented the best fit for the drying processes carried out at 30 and 50 °C, indicating that with this model, the changes in moisture content of the blueberries could be predicted with the drying time. However, the drying performed at 40 °C presented a worse fit to the proposed models, with data ranging between 0.9563 and 0.9573 for R^2, between 0.00112 and 0.00131 for χ^2 and between 0.03124 and 0.03157 for RMSE. In this case, the *Approximation of diffusion* model best described the characteristics of blueberry drying.

3.3. Determination of effective diffusivity and activation energy

The effective diffusivity was calculated from the slope of the line obtained by the natural logarithm of the moisture ratio versus the drying time using equation (8). As seen in Table 3, an increased temperatures produced increased diffusivity values from 2.58×10^{-11} at 30 °C to 4.58×10^{-11} m^2/s obtained at 50 °C, the same order of magnitude that some authors define for spherical products (Kaya, Aydm, & Dincer, 2010). These data showed that an increased temperature produced greater water diffusions within the fruit and a greater evaporation.

The activation energy was calculated from the slope of the line that formed the natural logarithm of the effective diffusivity against the inverse of the drying temperature (equation (11)). This parameter is defined as the energy needed to displace 1 mol of water from the sample to be dried. The blueberry variety studied presented an activation energy value of 23.13 kJ/mol, and other authors determined activation energy values between 36.2 and 54.5 kJ/mol in blueberry varieties (Vega-Gálvez et al., 2009), 57.85 kJ/mol (López et al., 2010) and 61.2 kJ/mol (Shi et al., 2008).

Table 2
Statistical analyses for different temperatures for drying processes of blueberries.

Model	Temperature (°C)	χ^2	RMSE	R^2
Approximation of diffusion	30	0.00031	0.01686	0.9907
	40	0.00112	0.03124	0.9573
	50	0.00034	0.01737	0.9882
Henderson and Pabis	30	0.00030	0.01686	0.9906
	40	0.00131	0.03130	0.9571
	50	0.00033	0.01737	0.9882
Page	30	0.00030	0.01672	0.9908
	40	0.00107	0.03136	0.9569
	50	0.00033	0.01736	0.9882
Two term	30	0.00032	0.01675	0.9907
	40	0.00118	0.03130	0.9571
	50	0.00036	0.01737	0.9882
Wang and Singh	30	0.00030	0.01696	0.9905
	40	0.00109	0.03157	0.9563
	50	0.00034	0.01758	0.9879

Table 3
Activation energy and effective diffusivity of drying processes of blueberries.

Activation energy (kJ/mol)	Temperature (°C)	Effective moisture diffusivity (m^2/s)
23.13 ± 5.40	30	$2.58 \times 10^{-11} \pm 2.04 \times 10^{-12}$
	40	$2.89 \times 10^{-11} \pm 5.59 \times 10^{-12}$
	50	$4.58 \times 10^{-11} \pm 4.07 \times 10^{-12}$

3.4. Spectrophotometric determinations

Color is an important parameter for measuring quality in fruits, and in the processed products derived from them, for example, in fruit juices, it is the first attribute that is perceived and can cause the acceptance or rejection of the food. Fig. 2 shows the absorbance values at 420, 520 and 620 nm, which are respectively related to the brown, red and blue-violet compounds of the obtained juices. All of the drying processes were maintained until the initial humidity was reduced by half; therefore, the concentration of the compounds inside the berries due to the evaporation of water should be the same in all cases. All of the juices from dried berries presented an increased absorbance values related to the J0 juice, with the juice from the blueberries dried at 50 °C presenting the highest absorbance value, this is because at higher drying temperature greater damage occurs in the blueberries skin and the compounds are released and diffused to the blueberries pulp. However, the hue values, which expresses the relation between the absorbance values at 420 and 520 nm (A420/A520), did not vary with temperature, obtaining a value of approximately 0.54 ± 0.02 in all

Table 1
Selected drying models for describing blueberry drying data.

Model name	Model	Reference
Approximation of diffusion	MR = a exp(-kt) + (1-a) exp(-kbt)	Yaldiz et al. (2001)
Henderson and Pabis	MR = a exp(-kt)	Henderson and Pabis (1961)
Page	MR = $\exp(-kt^n)$	Page (1949)
Two term	MR = a $\exp(-k_0 t)$ + b $\exp(k_1 t)$	Madamba et al. (1996)
Wang and singh	MR = 1 + at + bt^2	Wang and Singh (1978)

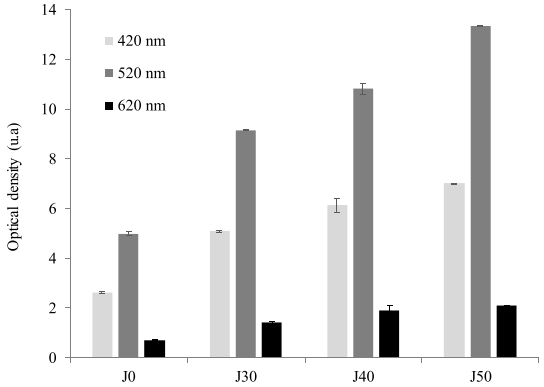

Fig. 2. Changes in the absorbances of blueberry juice after the drying process at 420, 520 and 620 nm.

cases, indicating that the juices obtained from both the undried and the dried fruits had a greater contribution of red-colored compounds compared to brown-colored compounds.

3.5. Total phenolic contents

Phenolic compounds are responsible for several of the health properties of the blueberries; they are found mostly in the skin of the fruits but also in the pulp (Hidalgo & Almajano, 2017). Table 4 shows the TPC values of the juices from undried and dried fruits at different temperatures. The drying process increased the content of phenolic compounds, which was higher at the highest temperature. After the statistical analysis it was found that significant differences existed between all the values. The final moisture content of the dried blueberries at each temperature was approximately the same, so that the concentration factor for the evaporation of water should be the same in all cases, as already discussed above. However, it can be verified that the drying performed at 50 °C was the temperature group with the highest content, obtaining 3.35 g GAE/L a value 3 times higher than the value of the juice from undried blueberries. On the other hand, drying at 30 and 40 °C increased the content of phenolic compounds 1.38 and 1.57 times, respectively, compared to the J0 value. This increase in phenolic compounds may be due to the fact that a higher temperature causes a greater diffusion of the compounds present in the skin into the blueberry pulp due to the cellular damage in the skin from the high temperature (Marquez et al., 2014; Marquez, Serratosa, & Merida, 2013). Other authors have demonstrated that the drying process at 45, 65 and 85 °C reduced the concentration of phenolic compounds in Bunchosia fruits, with greater decreases associated with the increased temperatures (Blank, Bellaver, Fraga, Lopes, & de Moura, 2018). In strawberries, a decrease in phenolic compounds of 60 and 78% has been observed during convective drying processes at 50 and 60 °C, respectively (Méndez-Lagunas, Rodríguez-Ramírez, Cruz-Gracida, Sandoval-Torres, & Barriada-Bernal, 2017). During a drying process, several mechanisms can occur that affect the concentration of phenolic compounds, such as the release of bound phenolic compounds and the partial degradation of lignin, leading to the release of phenolic derivatives and thermal degradation (Maillard & Berset, 1995). These reactions, which decrease the concentration of phenolic compounds, together with the concentration process due to the evaporation of water, result in a balance between the mechanisms that increase and those that decrease the concentration of the phenolic compounds.

Table 4
Effect of temperature on total phenolic contents (TPC) and antioxidant activity of juices from undried and dried blueberries.

Sample	TPC (g GAE/L)	Antioxidant activity (g Trolox/L)	
		DPPH assay	ABTS assay
J0	1.12 ± 0.02^{d}	0.63 ± 0.03^{d}	1.08 ± 0.02^{d}
J30	1.54 ± 0.05^{c}	0.79 ± 0.01^{c}	3.76 ± 0.04^{c}
J40	1.76 ± 0.01^{b}	0.90 ± 0.01^{b}	4.63 ± 0.04^{b}
J50	3.35 ± 0.05^{a}	1.04 ± 0.02^{a}	5.13 ± 0.05^{a}

Values in the same column with different superscript letters are significantly different, $p \leq 0.01$.

3.6. Anthocyanin concentrations

Anthocyanins are natural pigments present in the blueberry skins (Olas, 2018; Stevenson & Scalzo, 2012). The anthocyanin profile was determined in the juices from undried and dried blueberries (Fig. 3). In Table 5 it can be observed that fourteen compounds were identified in the juice from dried blueberries: the galactoside derivatives of malvidin, peonidin, petunidin, cyanidin and delphinidin; glucoside derivatives of malvidin, peonidin and cyanidin; arabinoside derivatives of malvidin, petunidin, cyanidin and delphinidin; and 2 aglicones (malvidin and cyanidin). Thirteen of the monomers mentioned were found in the undried blueberry juice as well. In all cases, the galactoside, glucoside and arabinoside derivatives of malvidin were the major compounds, malvidin-3-galactoside represented 42% of the total anthocyanins in the J0 juice and approximately 26% in the three dried blueberry juices. Malvidin 3-glucoside represented 22.9% in the fresh juice and approximately 18% in J30, J40 and J50 juices, while the arabinoside derivative represented 16.8% in the J0 juice and approximately 14% in the other three juices. Aglycones presented concentrations below 1 mg/L, and their content was very small in all of the juices tested. The drying process caused an increase in the concentration of anthocyanins, with the magnitude of the increase in anthocyanin concentration increasing with the temperature. The juice obtained from fruits dried at 50 °C presented the highest concentration of total anthocyanins (73.13 mg/L), 2.67 times greater than the concentration in the undried fruit juice. The increases in J30 and J40 were 1.90 and 2.18 times that of the J0 juice, respectively. Other authors have demonstrated that the drying processes at high temperatures causes a decrease in the anthocyanin contents of strawberries and blueberries (Méndez-Lagunas et al., 2017). As discussed above and considering the same effect of concentration, the temperature favors the diffusion of anthocyanins from the skin to the pulp. Furthermore, the drying process occurs at a moderate temperature, so thermal degradation reactions of the anthocyanins are not highly favored. On the other hand, anthocyanins are also degraded enzymatically in the presence of polyphenol oxidase (PPO), and these enzymes that produce oxidation of phenolic compounds can decrease their activity with increasing temperatures. Exists a balance between the mechanisms that increase and those that decrease the anthocyanin concentration, as previously mentioned.

3.7. Antioxidant activity

The antioxidant activity of the juices was determined by 2 methods, the DPPH and ABTS assays. As shown in Table 4, the increase in drying temperature produced an increase in the antioxidant activity values of the juices, significant differences existed between all values. The juice from fruits dried at 50 °C showed the highest antioxidant activity, 1.04 g Trolox/L and 5.13 g Trolox/L for the DPPH and ABTS assays, respectively. The results obtained by the different assays are not directly comparable due to the difference in the mechanisms of radical capture that occur in each method (Chaves et al., 2018). To evaluate the influence of phenolic compounds on antioxidant activity, a correlation study between the total phenolic compound values and the antioxidant activity was carried out, and the results are shown in Fig. 4. The coefficient of determination (R^2) for the DPPH method was 0.8073,

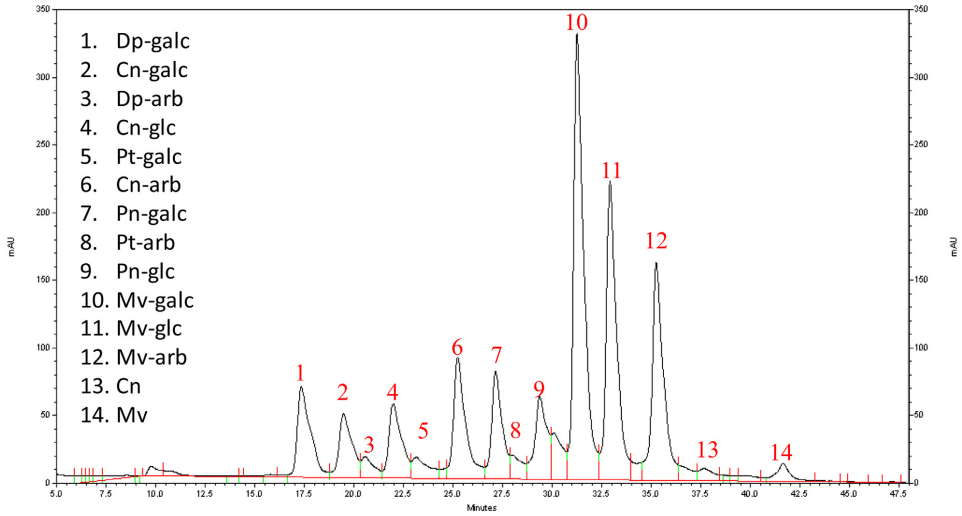

Fig. 3. Anthocyanins profile of the juice obtained from blueberry dried at 50 °C.

Table 5
Anthocyanin concentrations (mg/L) and homogeneous groups of juices from undried and dried blueberries.

Anthocyanins	J0	J30	J40	J50
Mv-galc	11.5 ± 0.09^{d}	13.9 ± 0.02^{c}	15.3 ± 0.02^{b}	19.0 ± 0.03^{a}
Pn-galc	0.54 ± 0.01^{d}	3.24 ± 0.03^{c}	3.97 ± 0.00^{b}	4.59 ± 0.01^{a}
Pt-galc	0.34 ± 0.01^{d}	0.89 ± 0.03^{c}	1.01 ± 0.00^{b}	1.27 ± 0.02^{a}
Cn-galc	n.d.	2.40 ± 0.02^{c}	2.99 ± 0.01^{b}	3.34 ± 0.04^{a}
Dp-galc	0.14 ± 0.01^{d}	2.91 ± 0.02^{c}	3.29 ± 0.02^{b}	4.77 ± 0.02^{a}
Total galactosides	12.6 ± 0.13^{d}	23.3 ± 0.12^{c}	26.6 ± 0.01^{b}	33.0 ± 0.07^{a}
Mv-glc	6.25 ± 0.06^{d}	9.75 ± 0.00^{c}	11.1 ± 0.03^{b}	12.8 ± 0.01^{a}
Pn-glc	0.63 ± 0.01^{d}	2.71 ± 0.04^{c}	3.21 ± 0.02^{b}	4.04 ± 0.03^{a}
Cn-glc	0.26 ± 0.02^{d}	2.36 ± 0.00^{c}	2.89 ± 0.02^{b}	3.78 ± 0.01^{a}
Total glucosides	7.13 ± 0.09^{d}	14.8 ± 0.04^{c}	17.2 ± 0.03^{b}	20.7 ± 0.03^{a}
Mv-arb	4.60 ± 0.00^{d}	7.34 ± 0.02 ^{c}	8.66 ± 0.03^{b}	10.1 ± 0.02^{a}
Pt-arb	0.53 ± 0.01^{c}	0.71 ± 0.02 ^{b}	0.75 ± 0.01^{b}	0.96 ± 0.04^{a}
Cn-arb	1.40 ± 0.04^{d}	4.07 ± 0.02 ^{c}	4.44 ± 0.01^{b}	6.00 ± 0.00^{a}
Dp-arb	0.67 ± 0.01^{b}	0.49 ± 0.01 ^{d}	0.57 ± 0.00^{c}	0.92 ± 0.00^{a}
Total arabinosides	7.20 ± 0.10^{d}	12.7 ± 0.07^{c}	14.4 ± 0.00^{b}	18.0 ± 0.06^{a}
Cn	0.09 ± 0.01^{c}	0.50 ± 0.04^{b}	0.51 ± 0.02^{b}	0.62 ± 0.02^{a}
Mv	0.37 ± 0.01^{d}	0.69 ± 0.00^{c}	0.83 ± 0.01^{b}	0.92 ± 0.01^{a}
Total aglycones	0.46 ± 0.00^{d}	1.19 ± 0.05^{c}	1.34 ± 0.03^{b}	1.54 ± 0.03^{a}
Total	27.4 ± 0.32^{d}	51.9 ± 0.27^{c}	59.5 ± 0.07^{b}	73.1 ± 0.08^{a}

Mv, malvidin; Pn, peonidin; Pt, petunidin; Cn, cyanidin; Dp, delphinidin; galc, galactoside; glc, glucoside; arb, arabinoside; n.d., not detected. Values in the same row with different superscript letters are significantly different, $p \leq 0.01$.

which indicates that there was a strong relationship between the antioxidant activity measured by this method and the content of phenolic compounds. The coefficient of determination for the ABTS method was 0.5671, which indicates that there may be other compounds that were not determined by the Folin-Ciocalteu method that interact with the radical $ABTS^{\cdot+}$ and have antioxidant capacities.

4. Conclusion

The increase of the drying temperature caused a decrease in the processing time up to 56% in the case of 50 °C with respect to drying at 30 °C. The processes of drying blueberries in a controlled temperature chamber is well suited to several kinetic models, with the *Page* model best describing the behavior of the blueberry moisture content in the drying at 30 and 50 °C, while the *Approximation of diffusion* model presented the best fit for drying at 40 °C. The effective diffusivity coefficient increased with increasing temperatures, which indicates that the temperature facilitates the diffusion of water through the fruit.

The final moisture ratio of the drying processes was 0.5, so the concentration effect of the fruit was the same in all cases. Despite this,

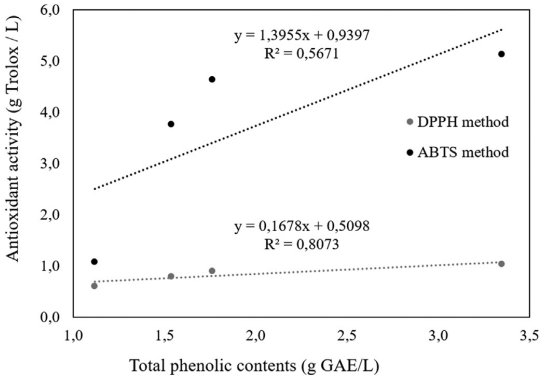

Fig. 4. Correlations between total phenolic contents and antioxidant activity values.

the color, the total phenolic compounds, the anthocyanin content and the antioxidant activity increased as the drying temperature increased. Drying at 50 °C yielded the highest values for the absorbances at 420, 520, and 620 nm, the highest content of phenolic compounds (3.35 g GAE/L), the highest concentration of total anthocyanins (73.13 mg/L) and the highest antioxidant activity with 1.04 g Trolox/L and 5.13 g Trolox/L for the DPPH and ABTS methods, respectively. There is a balance between the mechanisms that increase (such as the concentration effect by water evaporation) and those that decrease (such us the degradation reaction of phenolic compounds) the concentration of the compounds and the antioxidant activity values.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Juan Martín-Gómez: Conceived and designed the analysis, Collected the data, Contributed data or analysis tools, Performed the analysis, Wrote the paper. M. Ángeles Varo: Collected the data, Contributed data or analysis tools, Performed the analysis. Julieta Mérida: Conceived and designed the analysis, Wrote the paper. María P. Serratosa: Conceived and designed the analysis, Contributed data or analysis tools, Wrote the paper.

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