UNIVERSITAT ROVIRA I VIRGILI
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Aleksandra Rózek
ISBN:978-84-692-3227-9/DL:T-923-2009

Fruits and vegetables are increasingly being consumed because of their nutritional properties. The enrichment of these products with minerals, vitamins or physiologically active compounds that have potential benefits in terms of health maintenance and disease prevention may be a crucial way of developing new functional foods. Recently, extracts from grape seeds and skins have become a widespread nutritional supplement, because of their high content of phenolic compounds. These compounds have been shown to be highly bioavailable and to provide excellent health benefits. In the last few years, it has been suggested that osmotic treatment (OT) is a useful way of introducing controlled quantities of solution solutes into food and partially dehydrating it at the same time.

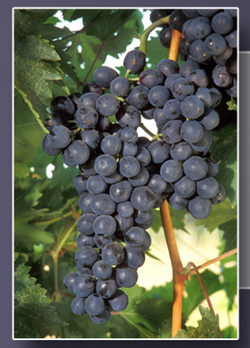
A range of solid foods enriched with grape phenolics from several sources were formulated using OT. Process parameters such as the source and concentration of the phenolic compounds, the nature and concentration of the osmo-active solute in the osmotic solution, and the food structure were investigated. Moreover the effect of a post-treatment such as convective air drying on the stability and antioxidant properties of the grape phenolics infused in the osmo-treated food was studied.

The results confirm that OT is a suitable technology for the exploitation of jelly foods, fruits and vegetables as matrices into which functional ingredients can be successfully incorporated to provide novel functional products of intermediate moisture. Concentrated red grape must and commercial grape seed and white grape marc extracts were successfully used as nutritional supplements. Their high content in phenolic compounds makes them ideal sources of natural antioxidants.

UNIVERSITAT ROVIRA I VIRGILI
Tarragona, 2009

Direct formulation of solid foods with grape phenolics

ALEKSANDRA RÓŻEK



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Doctoral Thesis
ALEKSANDRA RÓŻEK
Universitat Rovira i Virgili

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DOCTORAL THESIS

supervised by
Dr Montserrat Ferrando Cogollos

Department of Chemical Engineering

UNIVERSITAT ROVIRA I VIRGILI

Tarragona
2009

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Montserrat Ferrando Cogollos, Titular d'Escola Universitària del Departament d'Enginyeria Química de la Universitat Rovira i Virgili,

CERTIFICO:

Que el present treball, titulat "DIRECT FORMULATION OF SOLID FOODS WITH GRAPE PHENOLICS: STUDIES ON MASS TRANSFER AND ANTIOXIDANT CAPACITY", que presenta Aleksandra Różek per a l'obtenció del títol de Doctor, ha estat realizat sota la meva direcció al Departament d'Enginyeria Química d'aquesta universitat i que acompleix els requirements per poder optar a Menció Europea.

Tarragona, 7 de gener de 2009

Montserrat Ferrando Cogollos

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I am tempted to individually thank all of my friends which, from my graduate school until post-graduate school, have joined me in the discovery of what is life about and how to make the best of it. However, because the list might be too long and by fear of leaving someone out, I will simply say “thank you very much to you all”.
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I would like to thank to all my friends here in Tarragona, especially to *Vicente*, *Ronnie*, *Virginia*, *Luis* and *David* for standing by me in good and bad times, for all these nice moments, for their kindness and affection and for never letting me feel that I am away from my parents and country.

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Finally, it is needless to say that my thesis is still far from being perfect even though I have made my best. Though it is so, I expect that this thesis contributes benefits to all readers and those who are involved in this. For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own.

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TO MY PARENTS…

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NOTATION

A	absorbance
ABTS	2,2' –azinobis(3-ethylbenzothiazoline 6-sulfonate)
$ABTS^{\bullet+}$	radical cation
AD	air drying
a_w	water activity
CT	(+)-catechin
DAD	diode array detector
D_e	effective diffusion coefficient (m^2s^{-1})
DW	dry weight
ECT	(-)-epicatechin
ECG	(-)-epicatechin 3-*O*-gallate
EGCG	(-)-epigallocatechin 3-*O*-gallate
EGC	(-)-epigallocatechin
ET	single electron transfer
FCR	Folin-Ciocalteu's reagent
FRAP	ferric ion reducing antioxidant power
FW	fresh weight
GAE	gallic acid equivalent
GA	gallic acid
GSE	grape seed extract
HAT	hydrogen atom transfer
HPLC	High Performance Liquid Chromatography
k_1	Peleg rate constant (h), (s)
k_2	Peleg capacity constant (g/g)
$1/k_1$	initial rate (h^{-1}), (s^{-1})
$1/k_2$	equilibrium content (g/g)
LDL	low-density lipoprotein
M	molality (mol/L)
Mr	moisture ratio
ΔM	mass increment (kg/kg)
ΔN	gain in moles (mol/kg)
n	molar fraction (mol/kg)
n.s.	non significant
OD	osmotic dehydration
OS	osmotic solution
OT	osmotic treatment
ORAC	oxygen radical absorbance capacity
PA	protocatechuic acid
PAB1	procyanidin B1
PAB2	procyanidin B2
PBS	sodium phosphate buffer
PHr	individual polyphenol ratio
PR	penetration ratio
R	reduction (%)
R^2	regression coefficient
SE	standard error
t	processing time (h)

TEAC	Trolox Equivalent Antioxidant Capacity
TPH	total polyphenols
TPHr	total polyphenols ratio
TPTZ	2,4,6-tripyridyl-*s*-triazine
TRAP	total radical trapping antioxidant parameter
Y_r	diffusing substance ratio
Ψ (t)	moisture ratio during air drying
UV-Vis	ultraviolet visible
W	moisture concentration in the solid
WGME	white grape marc extract
x	mass fraction (kg/kg)
z	mass fraction in food liquid phase (kg/kg)

SUBSCRIPTS

0	initial
∞	equilibrium
a	half of the thickness of the cube
AD_0	initial conditions during air drying
AD_f	final conditions during air drying
c	critical content
e	equilibrium content
i	individual phenolic compound
j	each component
max	maximum
min	minimum
r	ratio
t	immersion time (h)

SUPERSCRIPTS

AD	air drying
GLY	glycerol
NaCl	sodium chloride
PHj, phj	each individual phenolic
ss	soluble solids
SUC	sucrose
TPH	total polyphenols
w	moisture

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RESUMEN

En la actualidad se está produciendo un incremento en el consumo de frutas y verduras debido a su elevado valor nutricional. El enriquecimiento de estos productos con minerales, vitaminas o compuestos fisiológicamente activos de los que se deriven efectos potencialmente beneficiosos para la salud y la prevención de enfermedades, puede resultar crucial en el desarrollo de nuevos alimentos funcionales. Todo esto ha dado lugar a un creciente interés en el uso de subproductos del procesado de alimentos como fuente de compuestos fisiológicamente activos (como los compuestos fenólicos) para enriquecer alimentos sólidos. Recientemente, los extractos de semillas y piel de uva se han convertido en un complemento nutricional muy extendido debido a su elevado contenido en compuestos fenólicos. Estos compuestos han mostrado ser altamente biodisponibles además de conferir excelentes beneficios a la salud. En los últimos años, la aplicación del tratamiento osmótico (TO) se ha propuesto como un método eficaz de introducir en el alimento cantidades controladas de los solutos presentes en la solución osmótica, deshidratándolo parcialmente. De esta forma, la composición del alimento y sus propiedades químicas y físicas pueden ser mejoradas. TO ha mostrado ser una técnica adecuada para incorporar ingredientes bioactivos en matrices tales como frutas, verduras y geles, dando lugar a una nueva categoría de alimentos funcionales y a nuevas oportunidades comerciales.

El principal objetivo del presente estudio ha sido formular alimentos sólidos de humedad intermedia enriquecidos con ingredientes bioactivos tales como compuestos fenólicos de uva mediante OT. En primer lugar, se empleó como solución osmótica (SO) un mosto concentrado de uva tinta. Se determinó el efecto de la concentración de solutos osmo-activos (azúcares) sobre i) el nivel de penetración de polifenoles de uva, ii) la ganancia de polifenoles de bajo peso molecular procedentes del mosto de uva y iii) la capacidad antioxidante. El alimento modelo (un gel de agar) fue procesado durante un tiempo máximo de 24 h y la presión osmótica se ajustó mediante dilución del concentrado de mosto de uva tinta. Tanto en el mosto como en el alimento tratado osmóticamente se cuantificó la concentración de los siguientes polifenoles: ácido trans-caftárico, ácido trans-coutárico, ácido ferulico, ácido cumárico, ácido cafeico (ácidos hidroxicinámicos), ácido gálico (ácidos hidroxibenzoicos), quercetina, y rutina (flavonol). Otros flavonoides como la (+) catequina y la (–) epicatequina (flavan-3-ols) únicamente fueron detectados

en el mosto concentrado. En los alimentos tratados osmóticamente se determinó TEAC (Trolox Equivalent Antioxidant Activity) y FRAP (Ferric Reducing Antioxidant Power). A las condiciones que maximizaron la penetración de polifenoles, el contenido en polifenoles totales del alimento modelo fue similar al encontrado en algunas frutas y verduras conocidas por su elevado contenido en polifenoles, mientas que los valores de TEAC fueron hasta tres veces superiores a los determinados en frutas con elevada capacidad antioxidante. Los resultados del análisis de regresión mostraron que los polifenoles de uva identificados explican significativamente el TEAC del alimento tratado osmóticamente.

Se caracterizó la transferencia de materia de los compuestos minoritarios presentes en la solución osmótica durante el TO con un mosto concentrado de uva tinta. A partir de la solución analítica de la segunda ley de Fick y del modelo de Peleg, se calcularon los coeficientes de difusión y las constantes de Peleg del agua, los polifenoles totales y los de algunos polifenoles de bajo peso molecular durante el TO del alimento modelo con el concentrado de uva tinta. Se detectó una transferencia significativa de polifenoles totales y polifenoles de bajo peso molecular (164 – 610 g/mol) durante el TO del alimento modelo con mosto concentrado de 40, 50 and 60 % (p/p) de sólidos solubles. Valores de concentración de sólidos solubles en el mosto superiores al 50% (p/p), disminuyeron de manera significativa la penetración de polifenoles totales e individuales. El peso molecular de los polifenoles también tuvo un importante efecto en la velocidad de impregnación. Según los resultados obtenidos, la penetración de polifenoles con un peso molecular superior a 600 g/mol contribuyó de forma limitada a la impregnación total de polifenoles.

Con el fin de formular una amplia gama de productos enriquecidos con polifenoles pero con significativas diferencias en su composición, se investigó el uso de otras fuentes de polifenoles distintas al mosto concentrado. En particular, se ha determinado la influencia de la concentración de polifenoles y del perfil fenólico de la SO, así como de la presión osmótica (actividad de agua), ajustada mediante la concentración de sacarosa, en la composición y propiedades antioxidantes del alimento tras el TO. Para ello, se emplearon como fuente de polifenoles dos extractos comerciales de uva obtenidos a partir de semillas (GSE) y orujo (WGME) con diferencias en su perfil fenólico como fuente de polifenoles mientras que el contenido en sacarosa de la SO fue del 50% y del 0% para obtener, respectivamente, un elevado y bajo gradiente de presión osmótica entre la SO y el alimento modelo. En relación a los polifenoles en el alimento tratado

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osmóticamente, se obtuvo una ganancia significativa tras 8 h de tratamiento con 50% de sacarosa y 15 g/L de polifenoles totales en la SO: se determinaron 7176 ± 52 mg GAE/kg and 6458 ± 32 mg GAE/kg en el alimento tratado con GSE y WGME, respectivamente. Cuando no se empleó sacarosa en la SO, el contenido en polifenoles totales del alimento modelo fue aproximadamente el doble del obtenido con 50% de sacarosa en la SO. Según esto, el contenido en sacarosa de la OS afectó a la infusión de polifenoles en mayor medida que el tipo de extracto empleado. Asimismo, el perfil fenólico del alimento tratado osmóticamente estuvo directamente determinado por la composición del extracto de uva empleado. En el alimento modelo tratado se encontraron polifenoles de bajo peso molecular pertenecientes a los siguientes grupos: ácidos hidroxibenzoicos, monómeros y dímeros de flavan-3-oles. Los monómeros de flavan-3-oles fueron los que se encontraron en concentraciones más elevadas, al tratarse de los mas abundantes en GSE y WGME. Durante el TO, la capacidad antioxidante del alimento tratado aumentó linealmente con el contenido en polifenoles.

Con el fin de describir la transferencia de materia de los solutos presentes en la SO, se empleó la solución de la ecuación de Fick para geometría cúbica y el modelo de Peleg. Los coeficientes de difusión de los polifenoles totales e individuales fueron inferiores a los coeficientes de difusión del agua y la sacarosa. El mayor coeficiente de difusión fue el de los polifenoles totales seguido del del ácido gálico ($0.90\ 10^{-10}$ m^2/s), del de los monómeros de flavan-3-ol (p.ej. catequina; $0.53\ 10^{-10}$ m^2/s), y del de la procianidina B1 ($0.22\ 10^{-10}$ m^2/s). Asimismo, durante el TO sin sacarosa en la SO los coeficientes de difusión de todos los polifenoles fueron significativamente mayores.

En relación al modelo de Peleg, se observó que la evolución del contenido en polifenoles del alimento modelo durante el TO se ajustó satisfactoriamente en todas las condiciones de operación consideradas (R^2>0.97). Según esto, la velocidad inicial de transferencia de polifenoles ($1/k_1$) y el contenido en polifenoles en condiciones de equilibrio ($1/k_2$) están determinadas por la concentración de polifenoles totales y de sacarosa en la SO. Tanto la velocidad inicial de transferencia de polifenoles como el contenido en polifenoles en condiciones de equilibrio aumentaron con el contenido en polifenoles totales de la SO. Se ha establecido que una concentración de 7.7 g GAE/L en la SO es suficiente para obtener una alimento osmo-deshidratado con un contenido en polifenoles similar al observado en frutas frescas ricas en polifenoles.

Para determinar como la naturaleza del soluto osmo-activo afecta la velocidad de transferencia de materia así como las propiedades antioxidantes de un alimento modelo

tratado osmóticamente, se emplearon sacarosa, cloruro sólido y glicerol como único soluto osmo-activo o en mezclas (sacarosa y cloruro sólido). De todos los solutos osmo-activos considerados, el cloruro sódico fue el que dio lugar a la mayor velocidad de penetración para cada uno de los polifenoles identificados. La transferencia de materia de los polifenoles totales y de los mayoritarios de bajo peso molecular, se caracterizó mediante un modelo difusional y el modelo de Peleg. La SO con cloruro sódico es la que dio lugar a los valores más elevados de coeficientes de difusión y de las constantes de Peleg $1/k_1$.

Se ha investigado el efecto del tipo de soluto osmo-activo, empleado individualmente o en mezclas, sobre el perfil de penetración de polifenoles en un alimento modelo. EL TO se llevó a cabo con una SO constituida por dos agentes osmóticos (NaCl y sacarosa) y un extracto comercial de pepita de uva. Para determinar cómo la composición de la solución osmótica afecta la penetración de los polifenoles en el alimento, la experimentación se planificó siguiendo un diseño experimental compuesto y central con dos factores (la molalidad de NaCl y sacarosa en SO). En todos los experimentos, se mantuvo constante la concentración de polifenoles totales en la SO (6300±45 mg GAE/kg) y el alimento modelo fue procesado durante 8 h. En toda la superficie de repuesta generada, se observó que el alimento modelo fue enriquecido en flavan-3-oles de forma significativa. En el punto central del diseño experimental, las concentración de los monómeros y dímeros de flavan-3-oles fue de 1334±126 y 486±55 mg/kg, respectivamente. Su penetración en el alimento modelo se vio limitada por la penetración de sacarosa. Se consiguió un TEAC en el alimento tratado osmóticamente superior al determinado en frutas conocidas por su elevada capacidad antioxidante.

Finalmente, se aplicó el TO para enriquecer en polifenoles tejido vegetal y evaluar la estabilidad de los polifenoles durante un tratamiento posterior como el secado convectivo por aire caliente. Para ello se trató osmóticamente un alimento modelo y tres alimentos de origen vegetal (dos frutas, plátano y manzana, y un tubérculo, la patata) que posteriormente fueron secados por aire caliente (55ºC). En la SO, se empleó cloruro sódico (10%, p/p) y sacarosa (50%, p/p) para tratar la patata y las frutas, respectivamente, mientras que un extracto comercial de semilla de uva fue la fuente de polifenoles (0.63%, p/p). En relación a la transferencia de polifenoles de la uva, el contenido en polifenoles totales y el TEAC se incrementó significativamente en todos los alimentos de origen vegetal. El TO, como pre-tratamiento, mostró un efecto protector frente a la degradación de los polifenoles de uva durante la etapa posterior de secado

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convectivo. El alimento modelo que fue tratado osmóticamente con la SO de control (sin soluto osmo-activo) mostró la mayor reducción en polifenoles totales (9.0%) seguida del plátano (3.7%) y de la manzana (3.7%).

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ABSTRACT

The model food (an agar gel) was processed for up to 24 hours and the osmotic pressure was adjusted by diluting the concentrated red grape must. In all the conditions tested, low molecular weight phenolics (≤610 g/mol), and in particular *trans*-caftaric acid, *trans*-coutaric acid, ferulic acid, coumaric acid, caffeic acid (hydroxycinnamic acids), gallic acid (hydroxybenzoic acids), quercetin, and rutin (flavonols), were quantified in the red grape must and also in the osmo-dehydrated food. TEAC (Trolox Equivalent Antioxidant Activity) and FRAP (Ferric Reducing Antioxidant Power) were determined in the osmo-dehydrated food. Under the conditions that maximized phenolic infusion, the total phenolic content of the gel was close to the values reported in some rich-in-phenolic fruits and vegetables while TEAC was three times that of fresh fruit with the highest antioxidant capacity. Regression analysis showed that the individual phenolics analyzed significantly explain the antioxidant capacity of the osmo-dehydrated food.

The mass transfer of minor solutes was evaluated in a multicomponent solution during OT of a model food with a concentrated red grape must. On the basis of the analytical solution to Fick's second law and Peleg's model, the effective diffusion coefficients and Peleg constants of water and solutes, including total phenolics and some individual phenolics of low molecular weight, were evaluated. A significant mass transfer of total phenolics and low-molecular-weight phenolics (164 – 610 g/mol) was detected during OD of a model food (an agar-agar gel) with a concentrated red must of 40, 50 and 60 % (w/w) soluble solids. When the soluble solids concentration of red grape must was increased to above 50 % (w/w), the penetration of total and individual phenolics in the model food significantly decreased. The molecular weight of the phenolics also had a significant impact on their infusion rate. The results showed that the penetration of phenolics whose molecular weight was above 600 g/mol might make a poor contribution to total phenolic impregnation.

To formulate a wide range of solid products supplemented with phenolics but with significant differences in their composition, other sources of phenolics were investigated. In particular, the influence of the phenolic profile and concentration of the osmotic solution (OS) and its osmotic pressure (i.e. water activity), adjusted for sucrose content, on the end composition and antioxidant properties of a solid model food after OT, was studied. To this end, two commercial grape extracts from seed (GSE) and white grape marc (WGME) with differences in their phenolic profile were used as a

source of phenolics when the sucrose content in OS was 50% and 0% to obtain, respectively, a high and low osmotic pressure gradient between the model food and OS. The phenolics in the osmo-treated food increased significantly after 8 hours of OT with 50% (w/w) sucrose and 15 g/L of total phenolics in OS: 7176 ± 52 mg GAE/kg and 6458 ± 32 mg GAE/kg were determined in the osmo-treated food with GSE and WGME, respectively. When sucrose was not present in the OS, the total phenolic content was almost twice that obtained with 50 % (w/w) sucrose in OS. Therefore, the sucrose content in OS affected phenolic infusion to a greater extent than the type of extract. The phenolic profile of the osmo-treated food was directly linked to the chemical composition of the grape extracts applied. Low molecular weight phenolics from the groups of hydroxybenzoic acids, flavan-3-ol monomers and dimers present in osmotic solution were also found in the osmo-treated food. The flavan-3-ol monomers were found in the highest concentrations, since they were the most abundant in GSE and WGME extracts. The antioxidant capacity of osmo-treated food increased linearly with the total phenolic content. In order to describe the mass transfer of solutes in multi-component OS during OT, the solutions to Fick's equation for cubical configuration and Peleg's model were used. The diffusion coefficients of total and individual phenolics were lower than the diffusion coefficients of water and sucrose. The diffusion coefficient of total phenolics was the greatest followed by the diffusion coefficients of gallic acid (0.90 10^{-10} m^2/s), flavan-3-ol monomers (i.e. catechin; 0.53 $10^{-10}$$m^2/s$), and procyanidin B1 (0.22 10^{-10} m^2/s). Besides, the diffusion coefficients of any phenolic were significantly higher during OT with non osmo-active solute. As far as Peleg's model is concerned, the experimental data of phenolic content during OT fitted well in almost all of the experimental conditions considered (R^2>0.97). The initial rate of phenolic mass transfer ($1/k_1$) and the phenolic equilibrium ($1/k_2$) in all the conditions was directly related to the concentration of phenolics and the presence of osmo-active solute in OS. Peleg's rate constant and Peleg's capacity constant of individual phenolics increased with the total phenolic content in OS. A total phenolic concentration of 7.7 g GAE/L in OS was sufficient to obtain an osmo-dehydrated food with a phenolic content similar to that found in fresh fruits that are rich in phenolics.

To investigate how the nature of osmo-active solutes affects the mass transfer of grape phenolics in a solid model food and its antioxidant properties, sucrose, sodium chloride and glycerol were used as single osmo-active solutes or mixtures (sucrose and sodium chloride). Of all the osmo-active solutes investigated, sodium chloride led to the highest

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phenolic infusion rate for each individual phenolic analysed. The mass transfer of total and individual grape phenolics of low molecular weight was characterized by the diffusional and Peleg models. The effective diffusion coefficients and $1/k_1$ Peleg constants of total phenolic model were highest with sodium chloride in OS.

How binary mixtures of osmo-active solutes affected the phenolic infusion pattern was investigated. OT was performed with an aqueous solution made up of osmo-active agents (NaCl and sucrose) and a commercial grape seed extract. Experimental conditions were set by a central composite design with two factors (the molality of NaCl and sucrose in the osmotic solution). In all the experiments the total phenolic content in the osmotic solution was kept constant (6300±45 mg GAE/kg) and the model food (an agar-agar gel) was processed for 8 hours. Throughout the response surface, the osmo-treated model food was significantly supplemented with flavan-3-ols. At the central point of the experimental design, flavan-3-ol monomers and dimers were found in concentrations of 1334±126 and 486±55 mg/kg, respectively. Their penetration into the model food was limited by sucrose to a different extent. The TEAC of the osmo-treated food was higher than that of fruits with a very high free radical scavenging activity.

Osmotic treatment (OT) was applied to infuse phenolic compounds into plant tissue and to evaluate their stability after a post-treatment such as convective air drying. A model food made of agar gel and three plant commodities (two fruits, apple and banana, and one vegetable, potato) were osmo-treated and subsequently air-dried (55ºC). In the osmotic solution, sodium chloride (10%, w/w) and sucrose (50%, w/w) were used when treating vegetables and fruits, respectively, while a commercial grape seed extract was the source of phenolics (0.63%, w/w). As far as the mass transfer of grape phenolics during OT is concerned, total phenolic content and antiradical scavenging capacity of plant foods were significantly increased. OT, as a pre-treatment, had a protective effect against grape phenolic degradation during further convective air drying, even though the mechanisms controlling the process require further research. The model food that was osmo-treated with the control OS (i.e. with a non osmo-active solute) showed the highest reduction in total phenolics (9.0%) followed by osmo-treated banana (3.7%) and apple (3.7%). OT of the model food with sucrose and sodium chloride OSs increased the total phenolic content. This was also observed in potato to a lesser extent.

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CHAPTER 1

PROJECT THESIS OVERVIEW

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1.1. STATE OF THE ART

1.1.1. FUNCTIONAL FOODS

In the last few decades consumer demands in the field of food production have changed considerably as consumers increasingly believe that foods contribute directly to their health (Mollet & Rawland, 2002). Foods today are not intended just to satisfy hunger and provide necessary nutrients but also to prevent nutrition-related diseases and improve consumers' physical and mental well-being (Menrad, 2003; Roberfroid, 2000). Interest has therefore grown worldwide in the research, development and commercialization of functional food ingredients, nutraceuticals and dietary supplements. Increasing demand for such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy and the desire of older people for improved quality of life in their later years (Roberfroid, 2000).

The term "functional food" was first used in Japan in the 1980s to refer to food products fortified with special constituents that have advantageous physiological effects (Stanton et al., 2005). Functional foods can improve the general conditions of the body (e.g. pre- and probiotics), decrease the risk of certain diseases (e.g. cholesterol-lowering products) and can even cure illnesses. As several demographical studies have shown, demand for these products exists as a result of the high cost of medical services for the aging population (Side, 2006).

"Functional foods" can be defined as "foods or ingredients of foods that provide an additional physiological benefit beyond their basic nutrition" (International Food Information Council, 2006; International Life Sciences Institute, 1999). Whole foods such as fruit and vegetables represent the simplest form of functional foods. Rich in bioactive phytochemicals such as polyphenols and carotenoids, they are known to have an antioxidant activity in protecting the body's cells against oxidative damage and to reduce the risk of developing certain types of cancer. Regardless of the official definition of "functional food", the demand from consumers for healthy foods has initiated an upsurge in research and product development in the food industry.

Most early developments of functional foods were those fortified with vitamins and/or minerals such as vitamin C, vitamin E, folic acid, zinc, iron, and calcium (Sloan, 2000). The focus later shifted to foods fortified with micronutrients such as omega-3 fatty acid,

phytosterol and soluble fibre to promote good health or to prevent diseases such as cancer (Sloan, 2004).

Functional foods have been developed in all food categories. From a product point of view, the functional property can be incorporated in several ways (see Table 1.1).

Table 1.1. Prominent types of functional foods (adapted from Siró et al., 2008)

Type of functional food	Definition	Example
Fortified product	A food fortified with additional nutrients	Fruit juices fortified with vitamin C
Enriched products	A food with added new nutrients or components not normally found in a particular food	Margarine with plant sterol ester, probiotics, prebiotics
Altered products	A food from which a deleterious component has been removed, reduced or replaced with another substance with beneficial effects	Fibres such as fat releasers in meat or ice cream products
Enhanced commodities	A food in which one of the components has been naturally enhanced through special growing conditions, new feed composition, genetic manipulation, or otherwise	Eggs with increased omega-3 content achieved by altered chicken feed

Functional food products are not homogenously scattered over all segments of the food and drink market and consumer health concerns and product preferences may vary between markets. These products have been mainly launched in the dairy-, confectionery-, soft-drinks-, bakery-, and baby-food markets (Kotilainen et al., 2006; Menrad et al., 2003). The most prominent types of functional products are presented briefly below:

- Probiotics—live microorganisms that, when consumed in sufficient quantities, confer a health benefit on the host;
- Prebiotics—non-digestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving the health of the host;
- Functional drinks—non-alcoholic beverages fortified with vitamin A, C and E or other functional ingredients. Other functional drinks are cholesterol-lowering drinks (with a combination of omega-3 and soy), "eye-health" drinks (with lutein) or "bone health" drinks (with calcium and inulin) (Keller, 2006);
- Functional cereals—cereals that can be used as sources of non-digestible carbohydrates and that, besides promoting several beneficial physiological effects, can selectively stimulate the growth of lactobacillus and bifidobacteria present in the colon and act as prebiotics. Cereals contain water-soluble fibre

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such as beta-glucan and arabinoxylan, oligosaccharides such as galacto- and fructo-oligosaccharides and resistant starch, which are reported to fulfil a prebiotic concept;

- Bakery products—products that contain the nutritional elements normally available in brown bread and include fibres, vitamins B1, B3 and B6, iron, zinc, inulin and a starch that comes from wheat (Benkouider, 2005);
- Spreads—those that contain phytostanol esters, intended to lower cholesterol levels, or camelina oil as a source of omega-3 fatty acids (Hopia, 2006);
- Functional meat—foods processed via the reformulation of fatty acid profiles or the inclusion of antioxidants, dietary fibre or probiotics, etc. (Ricondo & Ayo, 2007; Scollan, 2007);
- Functional eggs—those enriched with omega-3 fatty acids as well as with antioxidants, Se, vitamins D, E, B12 and folic acid, etc.

The technologies most frequently used in the development of functional food are (Fito and Chiralt, 2001):

a) improvements in traditional techniques for plant and animal production: for example, animal feed control;
b) genetically modified products;
c) physiologically active compounds mixed with traditional foods;
d) matrix engineering. This is a branch of food engineering that applies knowledge of food matrix composition, structure and properties to promote and control changes suitable for improving sensory and/or functional properties in food products.

Functional foods undoubtedly generate one of the most promising and dynamic segments of the food industry. In purchasing functional foods, consumers acquire a modern, positive impression of themselves. Such products provide consumers with a modern way in which to follow a healthy diet defined by nutrition experts. As attitudes to functional foods and their consumers are generally positive, this concept represents a sustainable trend in a multi-niche market.

1.1.2. ANTIOXIDANTS AND ANTIOXIDANT CAPACITY

In food systems, antioxidants normally refer to substances that in small quantities are able to prevent or greatly retard the oxidation of easily oxidizable materials such as fats (Chipault, 1990). In biological systems, the definition of antioxidants has been extended to "any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell, 1990). This latter definition covers all oxidizable substrates, i.e. lipids, proteins, DNA and carbohydrates. The main antioxidants are metal chelators (e.g. EDTA, preventive) and chain-breaking antioxidants (e.g. BHT, sacrificial) acting as hydrogen atom donors. In general there are four sources of antioxidants: (a) enzymes, e.g. superoxide dismutase, glutathione peroxidase, and catalase; (b) large molecules (albumin, ceruloplasmin, ferritin, and other proteins); (c) small molecules such as ascorbic acid, gluthatione, uric acid, tocopherol, carotenoids and polyphenols; and (d) some hormones (such as estrogen, angiotensin and melatonin, etc.). On the other hand, there are multiple free radical and oxidant sources (e.g. $O_2^{\bullet}$, $^1O_2$, $HO^{\bullet}$, $NO^{\bullet}$, $ONOO^-$, HOCl, $RO(O)^{\bullet}$, and $LO(O)^{\bullet}$). Oxidants and antioxidants have different chemical and physical characteristics. Antioxidants, for example, may respond differently to different radical or oxidant sources.

1.1.2.1. METHODS USED TO EVALUATE FREE RADICAL SCAVENGING ACTIVITY IN FOOD AND BIOLOGICAL SYSTEMS

Because of the complexity of food composition, separating each antioxidant compound and studying it individually is costly and inefficient, notwithstanding the possible synergistic interactions among the antioxidant compounds in a food mixture. It is very appealing to researchers, therefore, to have convenient methods for the quick quantification of antioxidant effectiveness in preventing diseases. However, such methods are yet to be developed. A total antioxidant capacity assay using one chemical reaction seems unrealistic but numerous methods have been published claiming to measure total antioxidant capacity in vitro. Frankel and Meyer (2000) reported that it is problematic to use one-dimensional methods to evaluate multifunctional food and biological antioxidants. They suggested that a general testing protocol should properly choose a biologically relevant substrate, test various oxidation conditions, measure both

initial and secondary oxidation products, compare antioxidants at the same molar concentrations of active components and quantify on the basis of induction period, percent inhibition, or IC_{50} (antioxidant concentration to achieve 50% inhibition).

Antioxidants can deactivate radicals by two major mechanisms. Depending on the reaction involved, two types of assays can be distinguished: (1) hydrogen atom transfer (HAT) reaction-based assays, and (2) single electron transfer (ET) reaction-based assays.

HAT-based assays measure the classical ability of an antioxidant to quench free radicals by hydrogen donating. Most HAT-based assays apply a competitive reaction scheme in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo-compounds. They generally comprise a synthetic free radical generator, an oxidizable molecular probe, and an antioxidant. HAT reactions are solvent and pH independent and are usually quite rapid. These assays include the inhibition of induced low-density lipoprotein autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocing bleaching assays.

ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour reduction is correlated with the sample's antioxidant concentrations. These assays involve one redox reaction with the oxidant (also as the probe for monitoring the reaction) as an indicator of the reaction endpoint and measure an antioxidant's reducing capacity. As ET-based assays are based primarily on the deprotonation and ionization potential of the reactive functional group, these assays are pH dependent. ET-based assays include the total phenols assay by the Folin-Ciocalteu's reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion-reducing antioxidant power (FRAP), total antioxidant potential assay using Cu(II) complex (as an oxidant), and DPPH.

HAT- and ET-based assays are intended to measure the radical scavenging capacity of a sample rather than the preventive antioxidant capacity. Each method generates a different radical through a variety of mechanisms.

1.1.2.1.1. ELECTRON TRANSFER BASED ASSAYS

These methods involve two components in the reaction mixture: antioxidant and oxidant (also the probe). They are based on the following electron-transfer reaction:

probe (oxidant) + electron (antioxidant) → reduced probe + oxidized antioxidant

The oxidant abstracts electrons from the antioxidant, which causes the probe to change colour. The degree of colour change is proportional to the concentration of the antioxidant. The reaction endpoint is reached when the colour stops changing. The change in absorbance is plotted against the concentration of antioxidant to produce a linear curve. The slope of the curve reflects the antioxidant's reducing capacity, which is expressed as Trolox equivalence or gallic acid equivalence.

The assays are carried out under acidic (FRAP), neutral (TEAC) or basic (FCR) conditions. The pH values have an important effect on the reducing capacity of the antioxidants. Applying multiple ET-based assays to measure the reducing capacity of an antioxidant often leads to excellent linear correlations between the results. Research papers on antioxidants have found excellent correlation ($R^2 > 0.99$) between total phenolic contents (measured by FCR) and antioxidant activity (measured by FRAP, TEAC, or DPPH assays). Because these assays are based on similar redox reactions, it is redundant to apply the multitude of assays in quantifying reducing capacity.

Total phenols assay by Folin Ciocalteu's reagent

This assay has become routine in the study of phenolic antioxidants. The method, developed in 1927, was used for tyrosine analysis (Folin, 1927), in which the oxidation of phenols by a molybdotungstate reagent yields a coloured product at 745–750 nm. Singleton and Rossi (1965) improved this method with a molybdotungstophosphoric heteropolyanion reagent that reduces phenols more specifically. Dissociation of a phenolic proton leads to phenolate anion, which is capable of reducing Folin-Ciocalteu's reagent. The blue compounds, formed between phenolate and reagent, are independent of the structure of phenolic compounds, which rules out the possibility of coordination complexes between the metal centre and the phenolic compounds. Generally, the results are expressed in gallic acid equivalents. Some papers, however, have replaced the recommended gallic acid standard with catechin equivalents (Vinson

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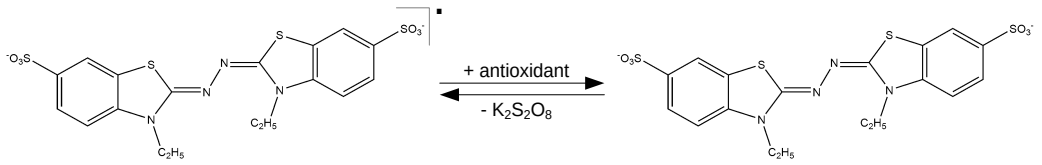
et al., 2001; Katsube et al., 2003), tannic acid equivalents (Nakamura et al., 2003), caffeic acid equivalents (Maranz et al., 2003), or ferulic acid equivalents (Velioglu et al., 1998).

Trolox equivalent antioxidant capacity (TEAC assay)

The TEAC assay, first reported by Miller and Rice-Evans (1993) and later improved by Re et al. (1999), is based on the scavenging ability of antioxidants to the long-life radical cation $ABTS^{\cdot+}$ (2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonate)) (Figure 1.1.). The original $ABTS^{\cdot+}$ assay was based on the activation of metmyoglobin with hydrogen peroxide in the presence of ABTS to produce the radical cation and in the presence or absence of antioxidants. It was reported that the faster-reacting antioxidants may contribute to the reduction of the ferryl myoglobin radical. In the improved version, the radical is generated directly in a stable form prior to reaction with putative antioxidants. The generation of $ABTS^{\cdot+}$ involves the direct production of the blue/green $ABTS^{\bullet+}$ chromophore through the reaction between ABTS and potassium persulfate.



$ABTS^{\bullet+}$ (λ_{max} = 734 nm) $ABTS^{2-}$ (colourless)

Figure 1.1. Free radical $ABTS^{\bullet+}$ in the presence of antioxidant

Adding antioxidants to the preformed radical cation reduces its ABTS to an extent and on a time-scale that depends on the antioxidant activity, the concentration of the antioxidant and the duration of the reaction. The method is applicable to the study of both water-soluble and lipid-soluble antioxidants, pure compounds, and food extracts.

Ferric Ion Reducing Antioxidant Power (FRAP assay)

The FRAP assay was originally developed by Benzie and Strain (1996) to measure reducing power in plasma but it was later adapted for the assay of antioxidants in botanicals. The ferric salt, $Fe(III)(TPTZ)_2Cl_3$ (TPTZ – 2,4,6-tripyridyl-*s*-triazine), is used as an oxidant and the reaction measures reduction to a coloured product. FRAP is conducted at acidic pH 3.6 to maintain iron solubility.

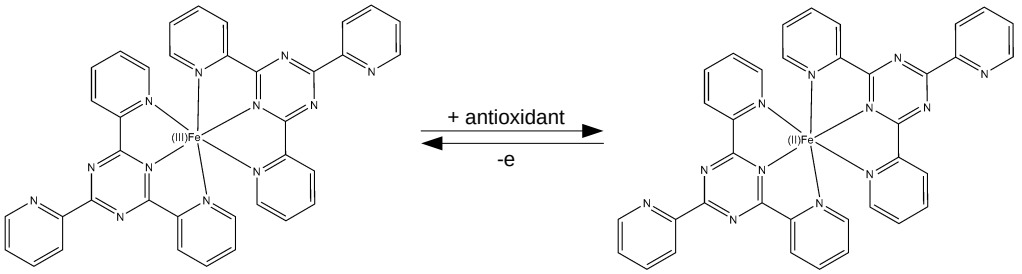


Figure 1.2. Free radical TPTZ in the presence of antioxidant

Reaction at low pH decreases the ionization potential that drives electron transfer and increases the redox potential, causing a shift in the dominant reaction mechanism (Simic and Jovanovic, 1994; Hagerman et al., 1998). The oxidant in the FRAP assay has a standard redox potential comparable to that of $ABTS^{2-}$ (~0.7 V). Except for the pH conditions, therefore, there is essentially little difference between the TEAC assay and the FRAP assay (Nillson et al., 2005).

A primary factor to consider in selecting a method concerns the reaction mechanism and its relationship to what might occur in the target application. For classical antioxidant action, an assay based on the HAT mechanism is preferred over one based on an ET reaction mechanism because the peroxyl radical is the predominant free radical found in lipid oxidation in foods and biological systems. ORAC, TRAP and LDL oxidation are considered to be the most biologically relevant assays. The antioxidant capacity from these in vitro methods may more closely reflect in vivo action. Prior et al., 2005 proposed that three of the most common methods (ORAC, TEAC and Folin-Ciocalteu's method) should be standardized for the routine quality control and measurement of the antioxidant capacity of dietary supplements and other botanicals since they have different reaction mechanisms (one uses the peroxyl radical because of its

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predominance in biological systems and the others use the electron transfer mechanism with $ABTS^{\bullet+}$ radical). These methods can measure both lipophilic and hydrophilic antioxidant capacity. Folin-Ciocalteu's method, TEAC and FRAP have been widely used in this study since they are commonly accepted and routinely practised in dietary antioxidant research laboratories throughout the world and because a large body of comparable data has been produced. The procedures are quite standardized and the reagents are commercially available. Moreover, the long-wavelength absorption of the applied reagents minimizes interference from the sample matrix, which is often coloured.

1.1.3. PHENOLIC COMPOUNDS AS FOOD ANTIOXIDANTS

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants (Randhir et al., 2004). These compounds, which form one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants, playing an important role in plant growth and reproduction, providing protection against pathogens and predators (Bravo, 1998), and contributing to the colour and sensory characteristics of fruits and vegetables (Alasalvar et al., 2001).

The importance of the antioxidant activities of phenolic compounds and their possible use as natural antioxidants in processed foods have reached new heights in recent years. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*- butylhyroquinone (TBHQ) have been widely used as food antioxidants but concerns over the safety of their use have encouraged interest in natural antioxidants (Balasundram et al., 2006). Synthetic antioxidants are substituted by phenolic compounds, so much of the research on natural antioxidants has also focused on these compounds (Martinez et al., 2002).

Grape extracts are commonly used to formulate dietary antioxidant supplements together with, for example, synthetic vitamins (E and C), minerals (selenium), soy isoflavones, tomato concentrate, rosemary extract, and citrus flavonoids (Dávalos et al., 2003; Monagas et al., 2006).

1.1.3.1. CLASSIFICATION OF PHENOLIC COMPOUNDS

Phenolic compounds have an aromatic ring bearing one or more hydroxyl groups and their structures range from simple phenolic molecules to highly polymerized compounds (Bravo, 1998). Most naturally occurring phenolic compounds are present as conjugates with mono- and polysaccharides linked to one or more of the phenolic groups. They may also occur as functional derivatives such as esters and methyl esters (Shahidi and Naczk, 1995). This structural diversity means that phenolic compounds can be divided into several groups, as shown in Table 1.2.

Table 1.2. Classes of phenolic compounds in plants (adapted from Balasundram et al., 2006)

Class		Structure
Simple phenolics, benzoquinones		C_6
Phenolic acids	Hydroxybenzoic acids	C_6-C_1
	Acethophenones, phenylacetic acids	C_6-C_2
	Hydroxycinnamic acids, phenylpropanoids (coumarins, isocoumarins, chromones, chromenes)	C_6-C_3
Napthoquinones		C_6-C_4
Xanthones		C_6-C_1-C_6
Stilbenes, anthraquinones		C_6-C_2-C_6
Flavonoids, isoflavonoids		C_6-C_3-C_6
Lignans, neolignans		$(C_6$-$C_3)_2$
Biflavonoids		$(C_6$-C_3-$C_6)_2$
Lignins		$(C_6$-$C_3)_n$
Condensed tannins (proanthocyanidins or flavolans)		$(C_6$-C_3-$C_6)_n$

This classification is based on: (a) the number of phenol rings the compounds contain, and (b) the structural elements connecting these rings. Phenolic acids, flavonoids and tannins are regarded as the main dietary phenolic compounds. These compounds, as well as stilbenes and lignans, are commonly found in grape berries. Brief descriptions of these five classes of phenolics are presented below.

Phenolic acids are acidic compounds because their structures contain one carboxylic group. These compounds include two subgroups, the hydroxybenzoic and hydroxycinnamic acids, derived from two non-phenolic compounds, benzoic and cinnamic acid, respectively. Hydroxybenzoic acids include gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids, which all have the C_6-C_1 structure. On the other hand, hydroxycinnamic acids are aromatic compounds with a three-carbon side chain (C_6-C_3). The most common hydroxycinnamic acids are caffeic, ferulic,

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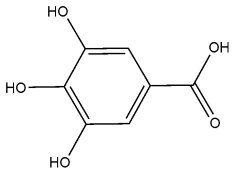
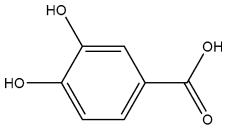
p-coumaric and sinapic acids (Bravo, 1998). Examples of hydroxybenzoic and hydroxycinnamic acids are shown in Figure 1.3.

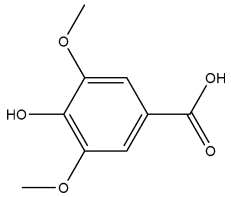
a)



Gallic acid

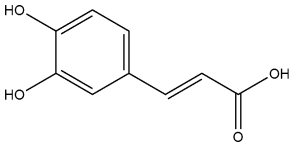


Protocatechuic acid

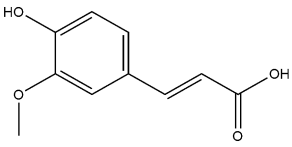


Syringic acid

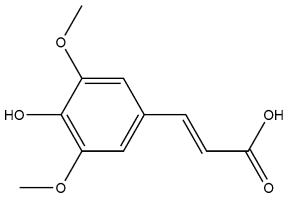
b)



Caffeic acid



Ferulic acid



Sinapic acid

Figure 1.3. Examples of a) hydroxybenzoic acids and b) hydroxycinnamic acids

Stilbenes are phenolic compounds that are present in the human diet only in low quantities because they are found either in plants that are not routinely consumed as food or in non-edible tissues. Hydroxylated stilbenes are found as glycosides in grapes and in a few other dietary sources. Stilbenes are produced by the grapevine in all tissues as a phytoalexin in response to a fungal attack such as that by *Botrytis cinera* (Waterhouse, 2002). Stilbenes are 1,2-diarylethenes, which contain a ring that usually carries two hydroxyl groups in the m-position, while the so-called B ring is substituted by hydroxyl and methoxy groups in the o-, m- and/or p-position (Figure 1.4.*)*. The principal stilbene in grapes is resveratrol. The anti-fungal compounds are the oligomers of resveratrol, which are called viniferins. Several forms of resveratrol exist, including the cis- and trans- isomers as well as the glucosides of both isomers (Waterhouse, 2002).

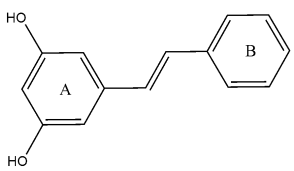


Figure 1.4. Molecular structure of stilben

Lignans are phenolic compounds ubiquitously distributed in plants but found in food in low quantities. They consist of two phenylpropane units (Figure 1.5.) and may be the source of phytestrogens in plant-rich diets.

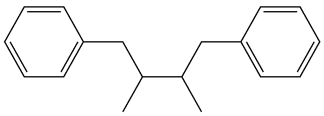


Figure 1.5. Molecular structure of lignan

Flavonoids are a large class of phenolic compounds widely distributed among plants and mainly found in cereals, fruits and vegetables (Naczk et al., 2006), medicinal plants (Araújo et al., 2008; Ali et al., 2008; Djeridane et al., 2006*)* and popular drinks such as juices (Vanamala et al., 2006; Caro et al., 2004), red wine, tea and beer. Flavonoids are low molecular weight compounds with a common structure consisting of two aromatic rings (A and B) joined by a 3-carbon bridge and an oxygen atom to form an oxygenated heterocyclic ring, C (Fig. 1.6.). The main flavonoid classes are the result of variations in substitution patterns to ring C based on: (a) the connection of one of the aromatic rings to the heterocyclic ring; (b) the oxidation state and functional groups of the heterocyclic ring (their oxidation level on the C-ring); and (c) the degree of polymerization. According to their chemical structure (Fig. 1.6), therefore, the main subclasses of flavonoids are flavonols, flavones, flavanones, isoflavones, anthocyanidins and flavanols (i.e. catechins).

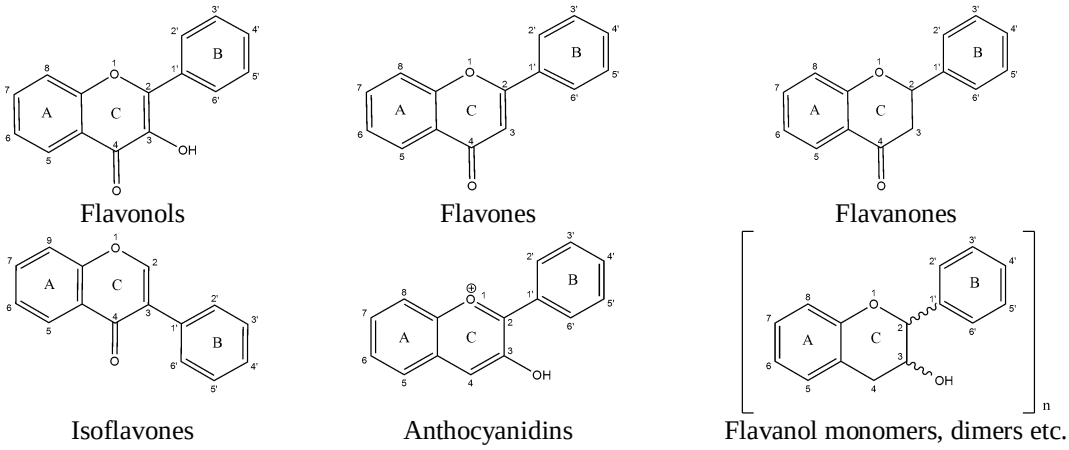


Figure 1.6. Generic structure of major classes of flavonoids

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In addition, substitutions to rings A and B give rise to the different compounds. These substitutions can include oxygenation, methoxylation, alkylation, glycosilation, sulfation and hydroxylation (Aherne et al., 2002). The biological activity of flavonoids depends on their chemical structure and on the relative orientation of various moieties on the molecule. Flavonoid glycosides are flavonoids with sugar moieties. The form that is not attached by sugar is called aglycone (Harborne, 1986). Flavanols are some of the most abundant subclasses of flavonoids often referred to as flavan-3-ols. These compounds are present as monomers, oligomers and polymers and are often esterified with gallic acid (Aherne, 2002).

Tannins are phenolic compounds with relatively high molecular weights and can be divided into condensed and hydrolysable tannins (Porter et al., 1989). *Condensed tannins* are mixtures of polymers of flavonoids (polyhydroxyflavan-3-ol monomers), while *hydrolysable tannin* are gallic- or elagic-acid-based mixtures, also called gallotannins or ellagitannins, respectively. Phlorotannins represent the third subdivision. Not significant in human diet, they consist entirely of phloroglucinol.

Though phenolic compounds are present in almost all foods of plant origin, fruits, vegetables and beverages are their main sources in the human diet (Chun et al., 2005). The level of these compounds in plant sources depends on factors such as cultivation technique, cultivar, growth conditions, ripening process, and processing and storage conditions. The content of some phenolics can increase under stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air pollution and exposure to extreme temperatures (Naczk and Shahidi, 2006). Several authors have reported wide variations in the total phenolic contents of different fruits and vegetables and even in the phenolic contents of the same fruits and vegetables. The differences may be due to the complexity of these compounds and to the methods of extraction and analysis (Bravo, 1998; Kalt et al., 2001). The phenolic contents of selected fruits and vegetables are shown in Table 1.3.

Table 1.3. Phenolics content of selected fruits and vegetables (adapted from Balasundram et al., 2006)

Fruit/Vegetable	Mean phenolic content in fresh mass (mg/kg)	Reference
Apple	2963 ± 64[a]	Sun et al., 2002
Banana	904 ± 32[a]	Sun et al., 2002
	118 ± 4[a]	Luximon-Ramma et al., 2003
Blackberry	4170 – 5550[a]	Sellappan et al., 2002
	267 – 4527[a]	Deighton et al., 2000
Blueberry (rabbiteye)	2700 – 9300[a]	Sellappan et al., 2002
Cherry	1054 ± 270[b]	Karakaya et al., 2001
Cranberry	5272 ± 215[a]	Sun et al., 2002
European elder	15400	Cieslik et al., 2006
Kiwi fruit	2730	Cieslik et al., 2006
Litchi	33.5 ± 0.5[c]	Gorinstein et al., 1999
	288 ± 17[a]	Luximon-Ramma et al., 2003
Mango	62.5 ± 0.5[c]	Gorinstein et al., 1999
	560 ± 21[a]	Luximon-Ramma et al., 2003
Nectarine	570	Cieslik et al., 2006
Orange	2170	Cieslik et al., 2006
Peach	846 ± 7[a]	Sun et al., 2002
Pink grapefruit	4250	Cieslik et al., 2006
Pink grapes	930	Cieslik et al., 2006
Papaya	576 ± 41[a]	Luximon-Ramma et al., 2003
Plums	1740 – 3750[a]	Kim et al., 2003
Red grape	2206 ± 612[c]	Karakaya et al., 2001
	2010 ± 29[a]	Sun et al., 2002
Strawberry	1610 – 2900[a]	Heinonen et al., 1998
	1600 ± 12.0[a]	Sun et al., 2002
White grapes	950	Cieslik et al., 2006
Zucchini	380	Cieslik et al., 2006
Tomato	620	Cieslik et al., 2006
	259 – 500[c]	Martinez-Valvedre et al., 2002
	680 ± 16[b]	Kaur et al., 2002
Carrot	1560	Cieslik et al., 2006
	564 ± 51[a]	Chu et al., 2002
	550 ± 9[b]	Kaur et al., 2002
Italian cabbage	1080	Cieslik et al., 2006
Onion	1500	Cieslik et al., 2006
	763 ± 19[a]	Chu et al., 2002
Broccoli	2900	Cieslik et al., 2006
	1016 ± 12.4[a]	Chu et al., 2002
	875 ± 81[b]	Kaur et al., 2002
Cabbage	546 ± 70[a]	Chu et al., 2002
	925 ± 24[b]	Kaur et al., 2002

[a] gallic acid equivalents
[b] catechins equivalents
[c] chlorogenic acid equivalents

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1.1.3.2. GRAPE PHENOLICS

Much research has been conducted to identify and quantify the main phenolic compounds of grapes. Grape seeds, skins and juice are known to contain a wide range of potent antioxidants in the form of polyphenols, which include phenolic acids, anthocyanins, and simple and complex flavonoids. The composition and content of polyphenols in grapes vary between cultivars and are influenced by geographical location and climatic conditions (Räthel et al., 2007). The quantity, structure and degree of polymerization of grape proanthocyanidins, for example, depend on their location in the grape tissues (Figure 1.7.).

Grape seeds contain higher concentrations (almost 20-fold more) of monomeric, oligomeric, and polymeric flavan-3-ols than grape skins (Monagas et al., 2003). Tannins in grape seeds tend to be in monomeric form rather than polymerised. Seed tannins consist mainly of epicatechin units as well as smaller amounts of catechin, epicatechin gallate, and epigallocatechin. Other compounds, such as quercetin 3-glucuronide followed by catechin, caftaric acid and astilbin, have also been detected in considerable amounts in seeds. Their quantity has been found to decrease considerably, following a second-order kinetic course, during ripening. The levels of gallates in the seeds are over 30% higher than in the skin and stems. Procyanidin B1 and B2 have also been detected in seed extracts, along with minor quantities of epigallocatechin and gallic acid (Guendez et al., 2005; Yilmaz & Toledo, 2004).

Catechin, epicatechin and epicatechin gallate are the main constitutive units of skin tannins, though gallocatechin and epigallocatechin are also present in minor quantities. Anthocyanins in grape skins, which are responsible for the red colour of grapes and wines, include delphinidin, cyanidin, petunidin, peonidin and malvidin 3-glucosides, 3-(6-acetyl)-glucosides and 3-(6-p-coumaroyl)-glucosides, peonidin and malvidin 3-(6-caffeoyl)-glucosides and some pyruvates. Quercetin and kaempferol glucosides and glucuronides, gallic acid and its glucosides, resveratrol, caftaric and coutaric acid complete the phenolic composition of grape skins.

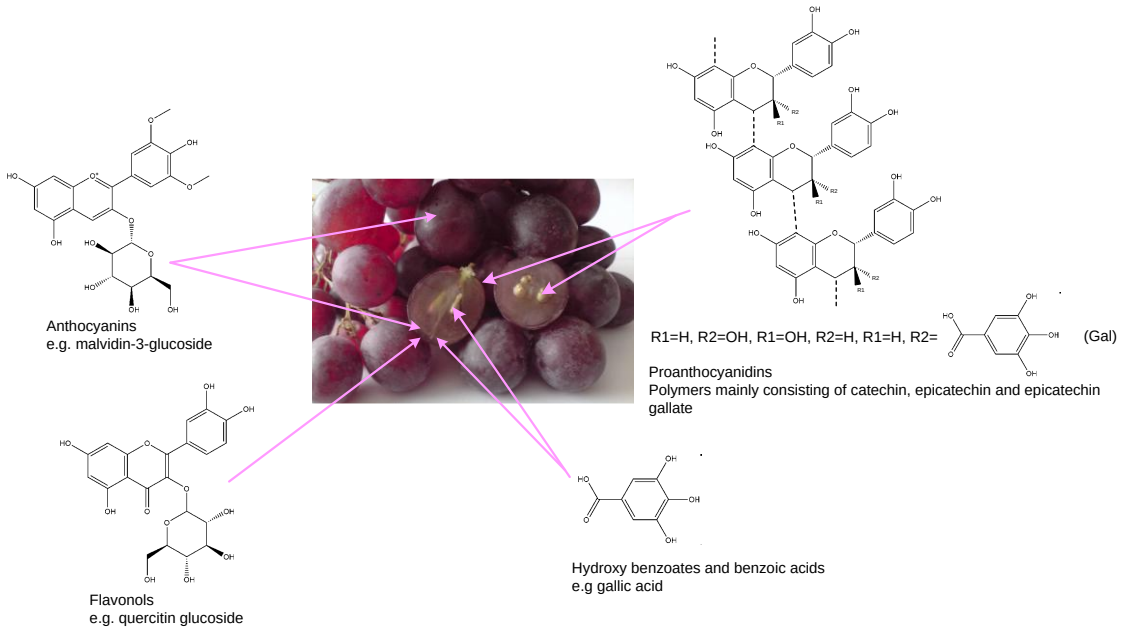

Figure 1.7. Cross-sectional picture of a red grape berry. Skin, pulp and seed can be distinguished (adapted from Pinelo et al., 2006).

Grape stems are also an attractive source of phenolic species. Wines made from non-destemmed grapes are known to generally contain higher levels of phenolic compounds than those prepared from destemmed grapes. Souquet et al. (2000) concluded that tannins are the most abundant phenolic constituents of grape stems (w/w, 80%).

1.1.3.3. RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND ANTIOXIDANT ACTIVITY OF PHENOLICS

The protective effects of phenolic compounds in biological systems are attributed to their capacity to transfer electron-free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases (Heim et al., 2002). This capacity is mainly related to the structure of the compounds. The antioxidant capacity of phenolic acids, for example, depends on the number and position of the hydroxyl groups in relation to the carboxyl functional group. The antioxidant capacity increases as the hydroxylation degree increases. For instance, trihydroxylated gallic acid shows a high antioxidant capacity. Substituting the hydroxyl groups at the 3- and 5-positions with methoxyl groups as in syringic acid reduces the activity (Rice-Evans et al., 1996).

Because of the relative complexity of flavonoid compounds, the relationship between their structure and antioxidant capacity is more complicated than it is for phenolic acids. Acker et al., 1996, reported that the hydroxylation degree and the positions of the –OH groups in the B ring results in higher stability to the aroxyl radical by electron delocalisation or acts as the preferred binding site for trace metals. The presence of hydroxyl groups at the 3'-, 4'-, and 5'- positions of ring B (a pyrogallol group) is reported to enhance the antioxidant activity of flavonoids in comparison with those with a single hydroxyl group (Acker et al, 1996). Also, in ring C, a double bond between C-2 and C-3, combined with a 3-OH, enhances antioxidant activity while substitution of the 3-OH decreases it. Pietta et al., 2000 reported that a double bond between C-2 and C-3, conjugated with the 4-oxo group in ring C, enhances the antioxidant capacity of flavonoids and that the substitution of hydroxyl groups in ring B by methoxyl groups alters the redox potential. It could also add another criterion. In the absence of an o-dihydroxy structure in the B-ring, hydroxyl substituents in a catechol structure on the A ring are able to compensate and become a larger determinant of flavonoid antiradical activity. According to van Acker et al. (1996), the basic flavonoid structure does not seem to be essential for good antioxidant activity. It only becomes important when the catechol moiety is not present. Moreover, the glycosilation of flavonoids largely decreases their antioxidant capacity.

1.1.3.4. EFFECT OF PHENOLIC COMPOUNDS ON HEALTH

Phenolic compounds are defined as bioactive compounds because they influence physiological or cellular activities, thus having a beneficial effect on health due to their cardioprotective, antiinflammatory, anticarcinogenic, antimutagenic, and other properties. These protective effects are related to their capacity to: (a) act as free radical scavengers; (b) chelate metals; (c) activate antioxidant enzymes; and (d) inhibit oxidases in biological systems. The most beneficial health effects attributed to flavonoids are due to their antioxidant and chelating properties. When phenolic compounds act as free radical scavengers, they protect the cell against oxidative stress and free radical-induced damage in membranes and nucleic acids. Antioxidant activity, probably the most widely studied aspect of the bioactivity of phenolic compounds, depends on the configuration and total number of hydroxyl groups in their structures. Several studies have attributed the cardiovascular protection to mechanisms that are not

related to their antioxidant capacity. These aspects, however are beyond the scope of the work, have been extensively reviewed by other authors (Chung et al., 1998; Khan et al., 2007; Tomera, 1999; Yilmaz et al., 2004).

1.1.3.5. BY-PRODUCTS OF THE FOOD INDUSTRY TO RECOVER PHENOLICS

The processing of plant foods leads to the production of by-products that are rich sources of bioactive compounds including phenolic compounds (Schieber et al., 2001). Their extraction and antioxidant properties are increasingly of interest (Moure et al., 2001) because of their numerous applications in the food industry. The citrus, olive, grape juice and wine-making industries, for example, produce large amounts of residues and wastes that are rich in phenolic compounds. Peel and seeds from the citrus industry can account for up to 50% of total fruit weight (Bocco et al., 1998). At the same time, the total phenolic content of lemon, orange and grapefruit peel is 15% higher than that of peeled fruits (Gorinstein et al. 2001). If an efficient extraction process is developed, therefore, citrus industry by-products could become a major source of phenolic compounds.

The by-products of the olive industry have also attracted considerable interest as a source of phenolic compounds. Much attention has focused on olive mill waste, particularly since the annual production exceeds 7 million tons (Ranalli et al., 2003). The phenolic content of olive mill waste water ranges between 1.0% and 1.8% (Visioli et al., 2003) depending on the variety and the process.

Grape seeds and skins, the by-products of grape juice and wine production, are also sources of several phenolic compounds, especially mono-, oligo-, and polymeric proanthocyanidins. Grapes are the world's largest fruit crop, with a reported annual production of over 42 million tons (FAOSTAT-FAO Statistical Database, 2005). The most important grape producers are France, Spain, Italy and the United States. Thanks to their beneficial health and lipid antioxidant properties, the recovery of phenolic compounds from grape by-products of the winemaking industry has recently attracted much interest (González-Paramás et al., 2004; Pinelo et al., 2005). The residues after fermentation, i.e. wine pomace, which mainly consists of grape seeds and skins, contain high levels of phenols, with most of the phenolic compounds retained in the skin matrix. These phenols are putative antioxidants, which justifies using grapes as a good source

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of phenol recovery (Kammemer et al., 2005). In Europe, 14.5 million tons of grape by-products are obtained from the wine industry every year.

The phenolic compounds obtained by the various extraction techniques are used as supplements and additives to beverages and other food items to provide a quantity of phenolics that is at least comparable to that of plant phenolics.

1.1.4. OSMOTIC TREATMENT AS AN ALTERNATIVE TO FOOD FORMULATION

Osmotic treatment (OT), also called osmotic dehydration or dewatering impregnation soaking, is a non-thermal treatment widely used to preserve and formulate food by modifying the composition of solid foods by partially removing water and adding solutes. By optimising the operating conditions, intermediate moisture foods enriched or fortified with a bioactive solute present in the osmotic solution can be formulated. OT also provides significant quality improvements in terms of colour, flavour, texture, packaging, distribution, cost, and energy efficiency, and ensures the required product stability and retention of nutrients during storage (Raoult-Wack, 1994, Rahman et al., 1999; Sablani et al., 2002). The direct formulation of solid foods and the improvement in their overall quality are distinctive advantages of this process over other dehydration methods.

During OT can be obtained a wide range of intermediate moisture products from fruits and vegetables such as apples (Sereno et al., 2001; Kaymak-Ertekin et al., 2000; Sacchetti et al., 2001; Derossi et al., 2008; Mavroudis et al., 1998; Barat et al., 2001; Moreira et al., 2003), bananas (Fernandes et al., 2006), pineapples (Rastogi et al., 2004), papayas (El-Aouar et al., 2006), orange (Cháfer et al., 2001), orange slices (Cháfer et al., 2003), potatoes (Eren et al., 2007; Colato et al., 2008), paprika (Ade-Omowaye et al., 2002), pumpkins (Mayor et al., 2006), tomatoes (Telis et al., 2004), and chestnuts (Moreira et al., 2007; Chenlo et al., 2006) among others.

OT is not a preserving method by itself since the typical final water activity of an osmo-treated food is in the 0.88 to 0.95 range. For this reason, OT has been used as a pre-treatment for further processing (Figure 1.8) such as freezing (Ponting, 1973), freeze drying (Hawkes and Flink, 1978), vacuum drying (Dixon and Jen, 1977) and air drying (Lewicki et al., 2000; Simal et al., 1997; Pereira et al., 2007).

Scientific research on the OT of foods was begun in 1966 by Ponting and co-workers. Since then a steady stream of publications has appeared. Many review articles have also been published (Le Maguer, 1988; Raoult-Wack, 1994; Raoult-Wack et al., 1992; Torreggiani, 1993) dealing with aspects such as the mechanisms underpinning OT, the effect of operating variables on OT, the modelling of water loss and solid gain etc. Since OT is an inherently slow process, several researchers have attempted to increase the mass transfer rates by combining OT with ultrasounds (Simal et al., 1998; Fernandes et al., 2008), γ-irradiation (Rastogi et al., 2006; Chetan et al., 2006), high pulsed electric field (Ade-Omowaye et al., 2003; Amami et al., 2007), vacuum (Fito et al., 1994; Rastogi et al., 1996), centrifugal force (Azuara et al., 1996; Amami et al., 2007) and high hydrostatic pressure (Rastogi et al., 2000).

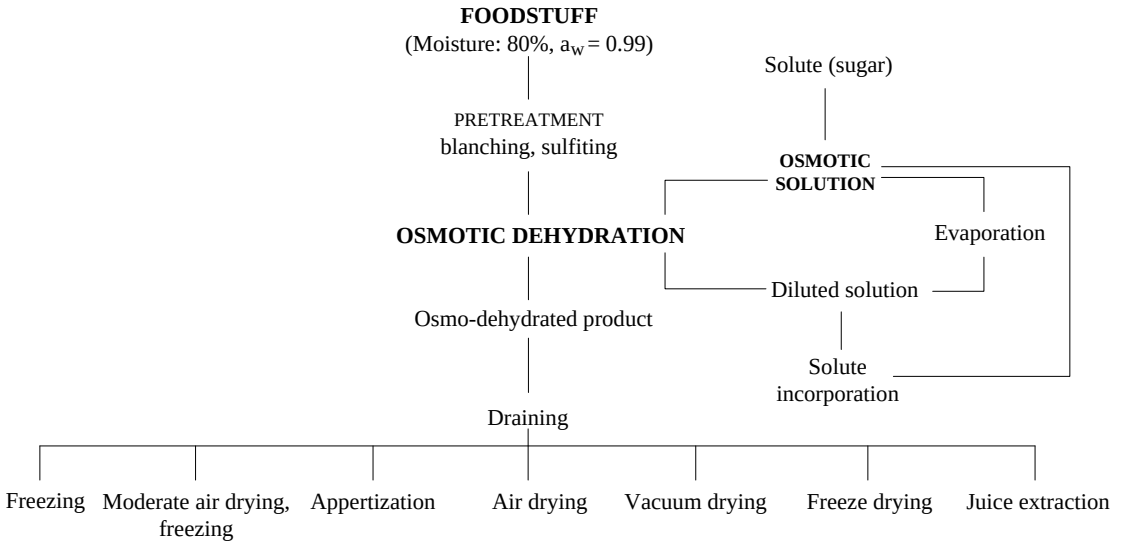

Figure 1.8. Applications of osmotic dehydration in fruit and vegetable processing (adapted from Torreggiani, 1993 and Spiess et al., 2001).

Although OT has been widely used to produce intermediate moisture products, it has been used only to a limited extent to produce functional foods from fruits and vegetables. Specifically, it has been used to impregnate plant food products with probiotics (Alzamora et al., 2005) and minerals (e.g. calcium and zinc) (Martin-Diana et al., 2007). In this study we investigate the suitability of using OT for infusing solid foodstuffs with grape phenolics.

1.1.4.1. PROCESS PARAMETERS

Several important parameters affect the mass transfer of water and solute(s) during OT. A key factor is the nature of the plant material, which is certainly influenced by possible pre-treatments. Torreggiani (1995) provided an example of the great variability in water and sugar exchanges observed between fruit of different species and between cultivars of the same species. This variability could be due to differences in soluble solid content, intercellular spaces, and other structural factors. The integrity of the tissue is essential for limiting solute uptake. Widely studied (Rastogi et al., 2002; Ade-Omowaye et al., 2003; Amami et al., 2007) has been the impact of other main process parameters, such as the pre-treatment methods (e.g. blanching, freezing, sulphitation, high pressure, high-intensity electric field pulses, freezing etc), the type and concentration of the osmo-active solute (e.g. sucrose, glucose and salt-sucrose mix), the temperature and working pressure (e.g. atmospheric pressure, vacuum, pulses of vacuum) on mass transfer and the physicochemical properties of many osmo-treated fruits and vegetables (e.g. colour and texture).

Any pre-treatment that modifies the tissue structure by disrupting cell membranes or the structural and chemical changes due to overripeness increases the impregnation effect during osmotic treatment (Heng et al., 1990). The composition of the concentrated solution is another key factor in OT. The choice of the type and concentration of the solute depends on several factors, namely the effects of its organoleptic quality properties, solubility, cell membrane permeability, stabilising effect and cost (Qi et al., 1998). The two most common solute types used in OT are sugars (mainly in fruits) and salts (in vegetables, fish, meat and cheese), particularly sucrose and sodium chloride (Ponting, 1973; Lenart et al., 1984). However, any very soluble solute or solvent that is miscible with water can be used (e.g. dextrose, starch syrup, ethanol, and mono- and polyols). Concentrated fruit juices have also been used to enhance the sensory properties of fruit products. Solutes can be used as dewatering and/or impregnating agents, requirements which are generally conflicting. Impregnation, for instance, is favoured by low-molecular-weight solutes while high-molecular-weight solutes promote dehydration. Blends comprising two or more solutes have been proposed since they may provide the advantages of both solutes (Raoult-Wack, 1994). Using ternary sucrose and sodium chloride aqueous solutions has several advantages in OT, such as higher levels of dehydration without over-salting the product and the possibility of

increasing the total solute concentration without attaining the saturation limit (Raoult-Wack, 1998). It is well known that increasing the concentration promotes mass exchange (Raoult-Wack, 1994; Torreggiani, 1993; Spiess et al., 2001). Water loss increases more than solid gain when the concentration of the solution increases.

When the processing temperature increases, the rate of mass transfer exchange increases, the viscosity of the solution decreases, solid-liquid contact is enhanced and processing time is reduced (Rastogi and Raghavarao, 1997). Osmotic treatments may also be carried out under vacuum and pulsed vacuum, leading to faster mass transfer kinetics, especially for tissue with a very porous structure (Fito & Chiralt, 2001). The rate of dehydration also increases as the level of agitation is increased. Agitation is indeed one of the key factors: an adequate level of agitation ensures the minimization or elimination of liquid-side mass transfer effects. As the OT progresses, the osmotic solution becomes increasingly diluted and the driving force for the further release of water drops. It is therefore necessary to have a solid-to-solution mass ratio of around 1:20 in order to minimize this dilution (Rastogi et al., 2002). Solid shape and size significantly affect water loss and solid gain, both of which increase as the ratio of the surface area to the characteristic length increases (Lerici et al., 1985).

In view of the above, the optimum process conditions depend on the particular application. For instance, candying requires high solid gain, which is favoured by osmotic solutes of low molecular weight at low concentrations.

1.1.4.2. MASS TRANSFER MECHANISMS DURING OSMOTIC TREATMENT

During OT, a driving force for water removal is set up because of a difference in osmotic pressure and water activity between the food and its surrounding solution. This creates two major simultaneous counter-current mass transfer fluxes (Torreggiani, 1993; Le Maguer, 1998), namely water outflow from the product to the surrounding solution and solute infusion into the product (Figure 1.9). A third type of mass transfer involving leakage of the product's own solutes occurs (sugars, organic acids, minerals, vitamins, etc.). This is quantitatively negligible compared with the first two types of mass transfer fluxes, but essentially with regard to the composition and sensory characteristics of the final product (Raoult-Wack, 1994). Overall, OT enables food water activity to be reduced and a controlled amount of an active principle, preservative agent, solute of

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nutritional interest, or sensory quality improver to be introduced concurrently into the end product.

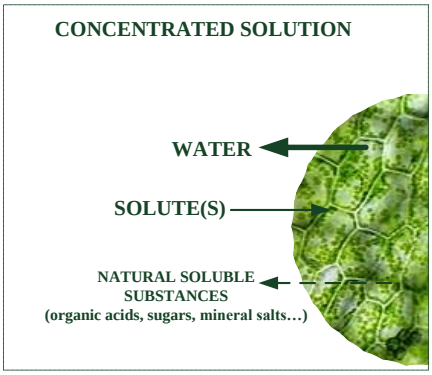

Figure 1.9. Schematic drawing of mass transfer during OT processes (adapted from Torreggiani, 1993 and Raoult-Wack, 1994)

Mass transfer during OT occurs through the semi-permeable cell membranes present in biological materials, which provide the dominant resistance to the process. The cell membranes can change from being partially permeable to being totally permeable, which can lead to significant changes in tissue architecture. The mechanisms of moisture transport during the OT of fruit and vegetable tissues are not fully understood. The most important pathways for mass transfer were proposed as apoplasmic transport (external to cell membranes) and symplasmic transmembrane flux (the water and solutes flow through intercellular channels due to differences in cell pressure) (Marcotte and Le Maguer, 1991). Rastogi et al. (2000) proposed that during the osmotic removal of water from foods, the OT front moves from the surface of the food in contact with the surrounding osmotic solution to the centre, which results in the disintegration of cells due to osmotic stress. Rastogi et al. (2000) therefore suggested that water is diffused across three regions (each with its own characteristic properties): Region I, from the core of the material to the dehydration front; Region II, the front; and Region III, from the osmotically treated material into the surrounding medium.

Several approaches have been used for modelling purposes. One of these is an empirical approach that uses mathematical equations fitted to experimental data to obtain mass transfer coefficients (Hawkes et al., 1978; Palou et al., 1994; Panagiotou et al., 1998; Peleg, 1988). Such models are simple to use but many experimental data are needed to obtain their empirical parameters. The semi-empirical approach takes into account several phenomena observed during the process but still has a strong empirical

component. Raoult-Wack, Petitdemange et al., (1991) proposed a bicompartmental model for simultaneous water and solute transport in agar gel cubes to explain the formation of a sucrose concentration layer on the surface of the product. In the prediction of concentration profiles during the osmotic dehydration of apples, Salvatori et al. (1999) proposed the concept of an "advancing disturbance front" that separates two zones: a zone of the tissue near the interface affected by mass transfer mechanisms with a developed concentration profile, and an undisturbed zone in which concentrations have not changed from their initial values. The distance between this front and the interface increases linearly with the treatment time. Both studies required the calculation of several experimental parameters.

Another, more fundamental, approach is based on the analysis of the mass transport phenomena that occur in the process. The simplest models consider only internal resistance to mass transfer and use Fick's second law (Equation 1.1) to obtain effective diffusion coefficients for the diffusing substances (Lazarides et al., 1997; Nsozy et al., 1998; Telis et al., 2004).

$$\frac{\partial C_f}{\partial t} = D_f \frac{\partial^2 C_f}{\partial x^2} \quad (1.1)$$

Another fundamental approach, which takes into account the thermodynamics of irreversible processes, is the Maxwell-Stefan approach for mass transfer in multicomponent systems (Wesselingh et al., 2000).

Other approaches are more complex and take into account different mass transfer mechanisms such as diffusion through membranes of different permeabilities, transport in intercellular spaces (by diffusion and/or convective movement), and occasional symplastic flow between cells (Conway et al., 1983; Magee et al., 1983; Marcotte et al., 1991; Touplin et al., 1986). These models are useful for describing mass transport phenomena at the cellular level but are perhaps too complex from a practical point of view.

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1.1.4.4. TECHNOLOGICAL APPLICATIONS OF OSMOTIC TREATMENT

Most industrial applications have been developed for plant material, mainly focused on producing intermediate moisture and dried tropical fruits products; of these, large volumes of finished products have been reported (Raoult-Wack, 1994).

More recently, osmotic treatments have been used for the production of fruit based food ingredients, such as pastry fillings, and fruit cubes dairy products. In Asian countries such as India and Thailand, the OT applied in the candying of tropical fruits is a popular fruit preservation method. For example, osmo-treated papaya, popularly known as 'tooti fruiti', is produced in small and medium-scale industries. In Europe (France and Italy, for example) advanced technologies have been developed to perform OT on an industrial scale.

Dehydrated fruits are gaining market as ready-to-eat snacks. The fruit slices can be sold as gift packs in line with dried nuts. Osmo-air-treated products that have lost about 70% of their moisture content can be consumed as snacks or shakes (after grinding and mixing with milk). Yadav et al., 2004 proposed OT process conditions for preparation of ready-to-eat snack from jack fruit, pineapple, banana, sapota and mango. To make the products shelf stable, the water activity was brought down considerably increasing the sugar levels by syrup treatment followed by hot air drying. The product packed in laminate pouches were stable for up to four months at ambient temperature. The process technology, of jack fruit and pineapple (Yadav et al., 2004) was transferred to the industry.

Osmotically dehydrated fruits were used directly in jam processing. Since the fruits (for example kiwi and orange) undergo a process at lower temperature (35-40°C) for short time during OT, the new jam product exhibits superior natural colour, good flavour and overall quality. Moreover sugar solution spent in OT can be used in jam (García-Martínez et al., 2002).

OT can be also applied as a pre-treatment in the deep-fat frying of potato strips (Krokida et al., 2001). French fries fried after OT had a better colour (less browning) and less oil uptake than those that were not treated and those that were pre-treated with air drying.

Furthermore, the number of industrial patents is an indicator of the interest in the applications of the treatment (Behsnilian et al., 1998). Some of them are outlined below:

- A formulation incorporating maltose or maltose corn syrup which allows increased levels of fructose or high fructose corn syrup to be infused into fruit or fruit pieces before drying. The formulation decreases the stickiness associated with high fructose infusion and agglomeration of fruit. Use of the formulation eliminates the need to oil coat fruit to keep it free-flowing (United States Patent 5690725),
- Production of crisp fruit or vegetable snack products including a sugar soaking step to prevent the collapse and shrinkage of the individual pieces of fruit or vegetable during the drying process. The method further comprises mixing the pieces of fruit or vegetable with a foam composition prior to drying (United States Patent 4889730),
- Treatment of kiwi fruit in buffered sugar solution prior to drying to stabilise the colour (United States Patent 5500241),
- The method for the improvement of the technical efficiency and of the quality of tinned mushrooms. This method is characterized by the fact that, in order to avoid blanching, the de-aeration of the mushrooms is achieved by treatment under a partial vacuum combined with an impregnation by means of a saline solution that makes a loss of water by osmosis possible (United States Patent 4680188),
- Pre-treatment for vacuum frying by immersion in solution of high osmotic pressure,
- Automatic continuous flow apparatus for drying fruits and vegetables, using sugar syrup process for slices, pieces or whole foods.

However, it seems quite limited development for a technique which has proven to be useful to improve the overall quality of food products in a wide range of applications. One reason may be the difficulty in developing continuous processing equipment and the management of spent solution, which gets diluted. The recycling of the spent solution requires it to be concentrated by evaporation, addition of solute or membrane processes etc.

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1.2. OBJECTIVES

The main aim of this study was to formulate intermediate-moisture solid foods with functional ingredients such as grape phenolics using OT. To satisfy this main objective, we also:

- evaluated how the source of phenolics, i.e. concentrated red grape juice and commercial extracts from grape seed and white grape marc, affects: (i) the penetration level of grape phenolics, (ii) the intake of low-molecular-weight phenolics, and (iii) antioxidant capacity in a model food made of agar during OT;

- characterised the mass transfer rate of low-molecular-weight phenolics during OT in a model food with each source of grape phenolics at different operating conditions. To do so, we investigated Fickian and empirical models;

- determined how the nature of the osmo-active solute (e.g. sucrose, sodium chloride, and glycerol) influences the mass transfer of grape phenolics in a model food and its antioxidant properties;

- evaluated how using different types of binary mixtures of osmo-active solutes (sucrose and sodium chloride) affects the phenolic pattern and antioxidant capacity of the final product;

- established the extent and rate of phenolic infusion into plant tissue (apple, banana and potato) and evaluated their stability after a post-treatment such as convective air drying.

1.3. CONCLUSIONS

We formulated a range of solid foods enriched or fortified with grape phenolics from several sources using OT. We studied process parameters such as the source and concentration of the phenolic compounds, the nature and concentration of the osmo-active solute in the osmotic solution, and the food structure. We also studied how a post-treatment such as convective air drying affected the stability and antioxidant properties of the grape phenolics infused in the osmo-treated food.

Our results confirm that OT is a suitable technology for the exploitation of jelly foods, fruits and vegetables as matrices into which functional ingredients can be successfully incorporated to provide novel functional products of intermediate moisture. Concentrated red grape must and commercial grape seed and white grape marc extracts were successfully used as nutritional supplements. Their high content in phenolic compounds makes them ideal sources of natural antioxidants that provide excellent health benefits.

According to results obtained during osmotic treatment with concentrated red grape must (Chapters 2 and 3), the concentration of osmo-active solute (sugars) in the osmotic solution (OS) significantly affected the penetration level of grape phenolics. Increasing the concentration of soluble solids of red grape must to over 50% (w/w) significantly decreased the penetration of total and individual phenolics in the model food. The effective diffusion coefficients of total and individual phenolics showed that not only the concentration of soluble solids in the osmotic solution but also the molecular weight of grape phenolics controlled their rate of infusion in the model food. The penetration of phenolics with a molecular weight of over 612 g/mol was lower and made a poor contribution to total phenolic impregnation. Using concentrated red grape must as osmotic solution enabled us to infuse low molecular phenolic compounds such as *trans*-caftaric acid, *trans*-coutaric acid, caffeic acid, coumaric acid, ferulic acid, gallic acid and flavonols such as rutin and quercitin. Regression analysis showed that the individual phenolics analyzed significantly explained the antioxidant capacity (measured by the FRAP and TEAC methods) of the osmo-dehydrated food. Under the conditions that maximized the phenolic infusion, the total phenolic content of the osmo-dehydrated model food was close to the values reported in some rich-in-phenolic fruits

and vegetables, while the TEAC was three times that of fresh fruit with the highest antioxidant capacity.

Using two commercial grape extracts from seed and white grape marc, we also studied how the phenolic concentration and profile, as well as the presence of osmo-active solute in OS, affected the rate of phenolic mass transfer during OT (Chapter 4). When sucrose was not present in the OS, the total phenolic content in the osmo-treated food was almost twice that obtained with 50% (w/w) sucrose in OS. This means that phenolic infusion was affected more by the sucrose content in OS than by the source of the extract. The phenolic profile of the osmo-treated food was directly linked to the chemical composition of the grape extract used. For all conditions tested, the hydroxybenzoic acid: gallic acid (GA), the flavan-3-ol monomers: (+)-catechin (CAT), (-)-epicatechin (ECT), (-)-epicatechin 3-*O*-gallate (ECG), (-)-epigallocatechin, (EGC), (-)-epigallocatechin 3-*O*-gallate (EGCG), and the flavan-3-ol dimers: procyanidin B1 (PAB1) and procyanidin B2 (PAB2) quantified in OS were determined in the osmo-treated food. When we compared the phenolic gain obtained with both grape extracts in the same conditions (total phenolic content and osmotic pressure of the OS), we found that the phenolic content in the osmo-treated model food was highest when grape seed extract (GSE) was used as the source of phenolics. These results may be explained by the differences in the individual phenolic composition between GSE and white grape marc extract (WGME). WGME is richer in flavan-3-ol dimers, whereas GSE contains more flavan-3-ol monomers, which, because of their lower molecular weight, penetrate the model food more during OT, causing differences in the profiles of individual phenolics in the osmo-treated model food depending on the source of phenolics used. Accordingly, CT, ECT and EGCT were found in greater concentrations in the model food osmo-treated with GSE, while the contents of the flavan-3-ol dimers, PAB1 and PAB2 were higher in the model food after OT with WGME. The mass transfer of solutes present in the multicomponent solution during OT was described using the solution to Fick's second law for cubical configuration and Peleg's model. Progress of total and individual phenolic contents during OT with no sucrose in OS fitted the diffusional model better, while the kinetics of individual phenolic infusion during OT with 50% sucrose in OS fitted Peleg's equation better. Only the progress of flaval-3-ol dimers (PAB1 and PAB2) was not predicted or was poorly predicted by the models

studied. Overall, the diffusion coefficients of individual phenolics were significantly higher during OT with non-osmo-active solute.

The nature of the osmo-active solute affected the mass transfer rate of grape phenolics in a solid model food and its antioxidant properties (Chapter 5). Sucrose, sodium chloride and glycerol were used as a single osmo-active solute or as a mixture (sucrose and sodium chloride). Of all the osmo-active solutes investigated, sodium chloride (either as a single osmo-active solute or in combination with sucrose) led to the highest rate of phenolic infusion for each individual phenolic analyzed. This behavior could not be completely explained by differences in OS viscosity, and the ionic nature of sodium chloride seems to promote phenolic infusion during the OT of the model food. These results were confirmed by both the diffusional and Peleg's models: the highest effective diffusion coefficients and $1/k_1$ Peleg constants of total phenolics were obtained with sodium chloride in OS.

We also studied how using binary mixtures of osmo-active solutes affected the phenolic infusion pattern (Chapter 6). OT was performed using aqueous solutions made of osmo-active agents (NaCl and sucrose) and a commercial grape seed extract with a constant phenolic concentration. Experimental conditions were set by a central composite design with two factors (the molality of NaCl and sucrose in osmotic solution). In all experiments, the total phenolic content in the osmotic solution was kept constant (6300±45 mg GAE/kg) and the model food (an agar-agar gel) was processed for 8 hours. Throughout the response surface, the osmo-treated model food was significantly supplemented with flavan-3-ols. A range of osmo-treated model foods can be formulated with a very high content of flavan-3-ols (monomers and dimers) and a similar a_w but with very different contents of NaCl and sucrose. By using more than one osmo-active solute and adjusting the composition of the osmotic solution, OT can control not only the phenolics content but also the sensory properties of the end product. The penetration of grape phenolics into the model food was limited by sucrose to different extents. The TEAC of the osmo-treated gel was higher than that of fruits with very high free radical scavenging activities. The extent of phenolic infusion significantly increased the antiradical scavenging capacity of the osmo-treated gel, while all the flavan-3-ols detected and the hydroxybenzoic acid, GA, also made significant contributions.

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We used Osmotic Treatment to infuse phenolic compounds into plant tissue and evaluated their stability after a post-treatment such as convective air drying (Chapter 7). A model food made of agar gel and three plant commodities—two fruits (apple and banana) and one vegetable (potato)—were osmo-treated and then air-dried (55ºC). In the osmotic solution, sodium chloride (10%, w/w) and sucrose (50%, w/w) were used when treating fruits and vegetables, respectively. A commercial grape seed extract was the source of phenolics (0.63%, w/w). With regard to the mass transfer of grape phenolics during OT, the total phenolic content and antiradical scavenging capacity of plant foods significantly increased. The extent of grape phenolic impregnation was controlled by the food structure and the kind of osmo-active solute. Plant tissue showed a lower grape phenolic infusion than that of the model food. OT as a pre-treatment protected against grape phenolic degradation during further convective air drying, though the mechanisms controlling the chemical changes undergone by grape phenolics require further research.

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CHAPTER 2

*PHENOLIC PENETRATION LEVEL AND ANTIOXIDANT CAPACITY IN FOOD OSMO-TREATED WITH RED GRAPE MUST**

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2.1. INTRODUCTION

A high consumption of fruit and vegetables maintains human health and reduces the risk of disease, mainly because they contain phytochemicals that have antioxidant characteristics. Although data produced so far in relation to dietary antioxidants not only support but also challenge the antioxidant hypothesis (Stanner et al., 2004), there is still an increasing interest in producing novel foods supplemented with dietary antioxidants. Phenolics, plant secondary metabolites, are some of the most abundant antioxidants in fruits and vegetables and their recovery from the by-products of agricultural industries is a matter of increasing interest (Dimitros et al., 2006). More specifically, grape is one of the world's largest fruit crops and the press residues resulting from wine-making (that is seeds, skins and highly pressed must) are rich in phenolics and generated in huge amounts (Kammemer et al., 2004). Two phenolic-rich by-products can be differentiated: grape pomace that is made of the solid residues and concentrated must which is obtained by evaporation of the highly pressed must.

Phenolics, especially anthocyanins, have been extracted from grape pomace for decades and used as a natural food colorant. Currently, a wide range of grape pomace extracts has been put on the market. However, these extracts are usually a complex mixture of phenolic compounds and quantitative data about their phenolic content is not available. Recent studies have reported that the antioxidant and antimicrobial properties (Ozkan et al., 2004) of grape pomace extracts depend on the extraction conditions and the varieties of the raw material (Gonzalez-Paramas et al., 2004; Torres et al., 2002; Alonso et al., 2002).

The use of these grape pomace extracts as a source of functional compounds is still incipient and some applications have been suggested to make confections, fruit fillings, sauces, beverages and pasta products. However, all those examples involve the direct addition of the grape pomace extract as an ingredient or additive. No application has been reported in which a fresh solid foodstuff is impregnated.

Osmotic dehydration (OD) is a well-known operation in food technology that enables water to be removed from the product and its functional properties to be modified by impregnation with particular solutes. OD commonly takes place by immersing the product in an aqueous solution with a greater osmotic pressure (i.e. with a relatively high concentration of dissolved substances, mainly sugars and salts). This creates two major simultaneous countercurrent mass transfer fluxes: namely, water outflow from the

product to the surrounding solution and solute infusion into the product (Spiess et al., 1998). As a result of these two main flows, OD has a double effect on solid foodstuffs: it partially removes the water from the food and impregnates the food with the solutes of the osmotic solution.

The main objective of this study is to investigate the possibilities to increase the antioxidant capacity of a solid foodstuff by impregnation with phenolics from grape. Because of its dewatering and impregnating effects, osmotic treatment was considered as the process of choice and concentrated red must was used as osmotic solution and as the source of phenolics. In order to assess the use of OD to supplement a model solid food with antioxidants under standard processing conditions

- the penetration level of grape phenolics,
- the intake of low molecular weight phenolics from grape must and
- the impact of the increase in phenolics on the antioxidant capacity

are determined. On this basis, OD is investigated as the first step in developing products with a potentially high natural antioxidant capacity.

2.2. MATERIALS AND METHODS

2.2.1. Osmotic solution and model food

The concentrated red grape must (vars. *Bobal, Garnacha* and *Tintorera*) was supplied by Concentrados Palleja, S.L (Riudoms, Spain). The red grape must had a mass fraction of soluble solids of 65% and a pH of 3.5 and was used as an osmotic solution. Tartaric acid (1 g/L) was added to prevent pH changes.

As a model food an agar-agar gel was prepared with 4% (w/w) agar-agar (Scharlau, Spain), 9.6% (w/w) sucrose and distilled water. The mixture was heated to 95°C in a microwave oven until the agar-agar was completely dissolved. Gelation was achieved by cooling at room temperature. The gel was then stored at 6°±2°C prior to use.

2.2.2. Osmotic dehydration

The experimental set-up consisted of two parts: a basket in which the gel samples were placed and a vessel that was filled with the osmotic solution. The basket contained five shelves and guaranteed total immersion of the sample in the osmotic solution. Agitation was provided by a magnetic stirrer. About 150g of agar-agar gel cubes (1cm side) were

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weighed and placed in the OD basket. The prepared basket was submerged in 2.7 L of osmotic solution. The model food was processed for 1, 2, 4, 8, 12, and 24 hours and the osmotic pressure was adjusted by diluting the concentrated red grape must to 40%, 50%, and 60% of the mass fraction of soluble solids. A 14:1 solution:gel ratio (w/w) prevented changes in the solution concentration. During the experiment, temperature was maintained at 25º±2ºC and the set-up was covered to minimize the effect of light. After osmotic treatment, the gel cubes were removed from the solution, gently blotted with tissue paper and weighed. Each experiment was carried out in triplicate. All experiments were run under atmospheric pressure.

2.2.3. Determination of moisture and soluble solids content

The moisture content of fresh and osmo-dehydrated food was determined with the 934.06 AOAC gravimetric method (1998). The concentration of soluble solids in osmotic solutions and in osmo-dehydrated food was determined by the 932.14 AOAC refractometric method (1998).

2.2.4. Extraction of phenolic compounds from the osmo-dehydrated food

To determine the extent of phenolic impregnation in the gel after osmotic dehydration, a sequential extraction was carried out. A sample of crushed gel (2.5g) was extracted sequentially with 15 mL of methanol: water (50/50, v/v) and 15 mL of acetone: water (50/50, v/v) solutions, for 1 hour in each extraction solvent and at room temperature. Each extraction was carried out in triplicate.

2.2.5. Determination of total phenolic content

The total phenolic content of red grape must and gel extracts was determined with Folin-Ciocalteu's method (Singleton et al., 1965). The test sample (1 mL) was mixed with 50 mL of distilled water, 5 mL of Folin-Ciocalteu's reagent and 20 mL of 20% sodium carbonate solution. After 30 min, the absorbance at 750 nm was recorded. The results were expressed as gallic acid equivalents (mg GAE/kg on wet basis).

2.2.6. Determination of individual phenolics by HPLC

Phenolics were identified and quantified by HPLC (Hewlett-Packard (HP)/Agilent). An automatic injector, HP 1000, was used for the injection. A Supelcosil^{TM} LC-18 column

(25 cm × 4.6 mm), with a particle size of 5 μm and an injection volume of 100 μl was kept at 40°C. A constant flow rate of 1.5 mL/min was used with two solvents: solvent A, acetic acid in water at pH 2.60; solvent B, 20% solvent A mixed with 80% acetonitrile. Peaks were monitored by an HPLC system equipped with a diode-array detector and were identified by their retention times and spectra in comparison with external standards. A diode array UV-Vis detector (DAD) was used to choose the maximum absorbance for each group of compounds, to control peak purity, and identify the spectra of some phenolics (Betés-Saura et al., 1996). The concentrations of the phenolic compounds identified were measured using external standard curves. Those hydroxycinnamic acids for which standards were not available were identified using their spectra and retention time, as described by other authors (Betés-Saura et al., 1996). Calibration curves (standard area in absorbance versus concentration in mg/L) were performed over the range of concentration observed, except for caftaric acid (*cis*- and *trans*-) and coutaric acid (*cis*- and *trans*-), which were calibrated using a caffeic acid and coumaric acid standard, respectively.

Gallic acid, *trans*-caftaric acid, *trans*-coutaric acid, caffeic acid, coumaric acid, ferulic acid, rutin, quercetin, (+) catechin, and (-) epicatechin were purchased from Sigma-Aldrich (Steinheim, Germany). Results were expressed as mg of phenol per kilogram on wet basis.

2.2.7. Trolox Equivalent Antioxidant Activity Assay: TEAC

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) method is a decolorization assay applicable to both lipophilic and hydrophilic antioxidants (Re et al., 1999). The method is based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\cdot+}$, a blue-green chromophore with characteristic absorption at 734 nm, compared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble vitamin E analogue. The addition of antioxidants to the preformed radical cation reduces it to ABTS, and leads to decolorization.

Trolox (ACROS ORGANICS, Geel, Belgium) was used as the antioxidant standard. Trolox was prepared in phosphate buffered saline PBS, pH 7.4 (Sigma-Aldrich, Steinheim, Germany), for use as the stock standard. ABTS was obtained from Sigma-Aldrich (Steinheim, Germany), and potassium persulfate (potassium peroxodisulfate) from J. T. Baker (Deventer, Holland).

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ABTS was dissolved in water to a concentration of 7 mM. An ABTS radical cation (ABTS·+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. To study the antioxidant capacity of the extracts, the ABTS·+ solution was diluted with PBS, pH 7.4, to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30°C. The absorbance was verified by a Diode Array spectrophotometer (Hewlett Packard 8452A).

After 20 µl of extract or PBS had been added to 2 ml of diluted ABTS·+ solution (A_{734nm}=0.70±0.02), the absorbance reading was taken at 30°C exactly 5 minutes after initial mixing. Appropriate solvent blanks were run in each assay. All determinations were carried out in duplicate. The percentage inhibition was calculated using equation (2.1):

$$\Delta A_{(sample)} = \frac{A_{t=0\,\min(sample)} - A_{t=5\,\min(sample)}}{A_{t=0\,\min(sample)}} - \frac{A_{t=0\,\min(PBS)} - A_{t=5\,\min(PBS)}}{A_{t=0\,\min(PBS)}} \quad (2.1)$$

where $A_{t=0\ min}$ and $A_{t=5\ min}$ are the initial and the absorbance reading after 5 minutes. Percent inhibition values were obtained by multiplying $\Delta A_{(sample)}$ values by 100. The percentage inhibition was compared with the standard calibration curve for Trolox (R^2=0.999) and the results were expressed as the Trolox equivalent in mmol per kilogram on wet basis.

2.2.8. Ferric Reducing-Antioxidant Power Assay: FRAP

The method is based on reducing the Fe^{3+}-TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) complex to the ferrous form at low pH (Benzie et al., 1996). This reduction is monitored by measuring the absorption change at 593 nm. Electron donating substances whose half reaction has a lower redox potential than Fe^{3+}/Fe^{2+} - TPTZ drive the reaction and the formation of the blue complex forward.

The FRAP reagent was prepared as a mixture of 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ (Sigma Aldrich, Steinheim, Germany) in 40 mM hydrochloric acid at 50°C, and 20 mM ferric chloride (10:1:1, v:v:v). Every day 3 ml of working FRAP reagent was prepared and mixed with 100 µl of sample. The absorbance at 593 nm was recorded after 5 min incubation at 30°C on a diode array spectrophotometer (Hewlett Packard 8452A).

FRAP values were obtained by comparing the absorption changes in the test mixture with those when Trolox was used as a standard. Results were expressed as the Trolox equivalent in mmol per kilogram on wet basis. This procedure was used to analyse all solid gel extracts in duplicate.

2.2.9. Calculation procedures

The osmotic dehydration kinetics of the model food was evaluated by calculating the water loss ($-\Delta M^{w}$), soluble solid gain (ΔM^{SS}), and phenolic gain (ΔM^{TPH}). These parameters were calculated as:

$$\Delta M^{w} = \frac{M_t \cdot x_t^{w} - M_0 \cdot x_0^{w}}{M_0} \qquad (2.2)$$

$$\Delta M^{SS} = \frac{M_t \cdot x_t^{SS} - M_0 \cdot x_0^{SS}}{M_0} \qquad (2.3)$$

$$\Delta M^{TPH} = \frac{M_t \cdot x_t^{TPH} - M_0 \cdot x_0^{TPH}}{M_0} \qquad (2.4)$$

where M and x are the mass of the gel and the mass fraction of each component in the gel, respectively, the sub-indexes *0* and *t* indicate initial conditions and conditions at time t of treatment, and super-indexes *w*, *ss*, and *TPH* are water, soluble solids, total phenolics, respectively. From this point on, mass fraction of each component in the gel will be expressed as kg/kg on wet basis.

2.2.10. Statistical Analysis

For regression analysis, SPSS 13.0 was used.

2.3. RESULTS AND DISCUSSION

2.3.1. Phenolic profile of the concentrated red must (osmotic solution)

A concentrated red must with a soluble solid mass fraction of 64.5%, coming from a mixture of three grape varieties, was used as a source of phenolics in the osmotic solution. Table 2.1 shows the content of soluble solids, total phenolics and the individual phenolics identified and quantified in the concentrated red must. The soluble solids concentration of the concentrated red must was 3 times higher than that of a fresh grape must.

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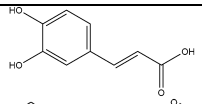
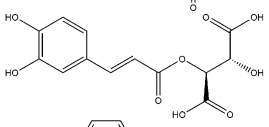
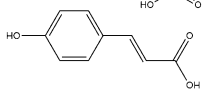
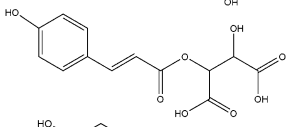
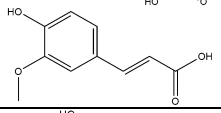
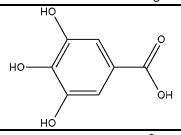
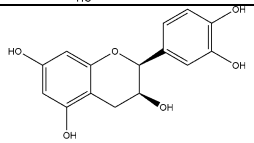
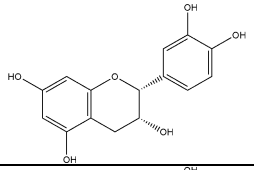
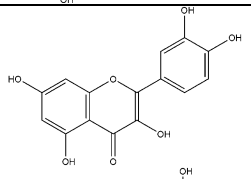
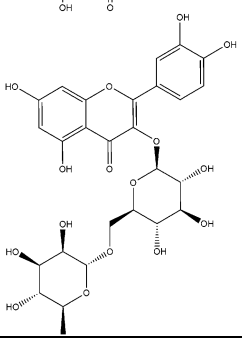
Table 2.1. Phenolic composition of the concentrated red must with a soluble solids mass fraction of 64.5% (mean ± standard deviation of determinations performed in triplicate)

	Concentration [mg/kg]	Molecular weight [g/mol]	Molecular structure	Phenolic classification
caffeic acid	11.8±1.8	180.20		Non-flavonoids: Hydroxycinnamic acids/ Hydroxycinnamates
trans-caftaric acid	147.2±5.8	312.23		
coumaric acid	29.8±1.4	164.16		
trans- coutaric acid	104.0±6.4	295.00		
ferulic acid	35.5±2.9	194.18		
gallic acid	318.7±13.2	170.12		Non-flavonoids: Hydroxybenzoic acids
(+)-catechin	66.7±2.4	290.28		Flavonoids: Flavan-3-ols
(-)-epicatechin	59.6±1.6	290.27		
quercetin	28.9±1.2	338.27		Flavonoids: Flavonol
rutin	77.5±1.6	610.53		
Total phenolics$_{FC}$[a]	13152±276	-	-	-
Total phenolics$_{HPLC}$[b]	879.7	-	-	-
Total hydroxycinnamics*	328.2	-	-	-
Total flavonoids*	232.8	-	-	-
Total benzoic acids*	318.7	-	-	-

[a, b] Total phenolics determined using the Folin-Ciocalteau method and HPLC, respectively. Total phenolics$_{FC}$ is expressed as mg GAE/kg
* Calculated from HPLC results.

The identification and quantification of grape phenolics was focused on those with a low molecular weight since molecular weight has been reported to slightly limit solute infusion during OD (Spiess et al., 1998). On this basis, hydroxycinnamic acids and their corresponding hydroxycinnamate and hydroxybenzoic acids were quantified from the group of non-flavonoids, while two flavan-3-ols and two flavonols were identified from the group of flavonoids. Although some anthocyanins might be extracted from grape skins during the production of the concentrated red must, they are not analyzed here as the work has focused on the intake of low molecular weight phenolics. Table 2.1 lists the concentration of the phenolics identified and quantified in the concentrated red must, together with their molecular weight and structure.

From the hydroxycinammates, *trans*-caftaric acid was found at highest level (147.2±1.7 mg/kg) followed by *trans*-coutaric acid (104.0±1.7 mg/kg). Fertaric acid was not detected. Ferulic acid was the free cinnamic acid detected in the highest concentration in the must (35.5±0.9 mg/kg), probably because of an extended hydrolysis of the tartrate ester, fertaric acid, which might explain why this ester was not detected. Coumaric acid and caffeic acid were the cinnamic acids found in the lowest concentrations (29.8±0.9 and 11.8±1.8 mg/kg, respectively). Gallic acid, the only benzoic acid detected, was present in an extremely high concentration (318.9±4.8 mg/kg), which is several orders of magnitude higher than the usual concentration in grape must (Betés-Saura et al., 1996). The hydrolysis of the ester forms of some flavonols produces free gallic acid. So, the operating conditions during the production of the concentrated grape must could lead to this high gallic acid concentration.

Flavonoids were present in lower concentrations than hydroxycinnamates and gallic acid; about 26% of the total phenols quantified by HPLC were flavonoids. Because the flavonoids quantified (flavan-3-nols and flavonols), are typically found in the grape seed and/or skin, they may be in the must as a result of the extraction method used to obtain the grape juice. Must was richer in (+)-catechin (66.7±0.3 mg/kg) than in (–)-epicatechin (59.6±0.5 mg/kg). Quercetin and rutin were the flavonols detected at an average level of 28.9±0.5 and 77.5±0.6 mg/kg, respectively.

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2.3.2. Effect of the operating conditions on water loss and soluble solid and phenolic gain

Figure 2.1 plots the effect of the soluble solids concentration of the osmotic solution on water loss, soluble solid and total phenolic gain during OD. As expected, water loss ($-\Delta M^{w}$) increased with time and also with the soluble solids concentration of the osmotic solution.

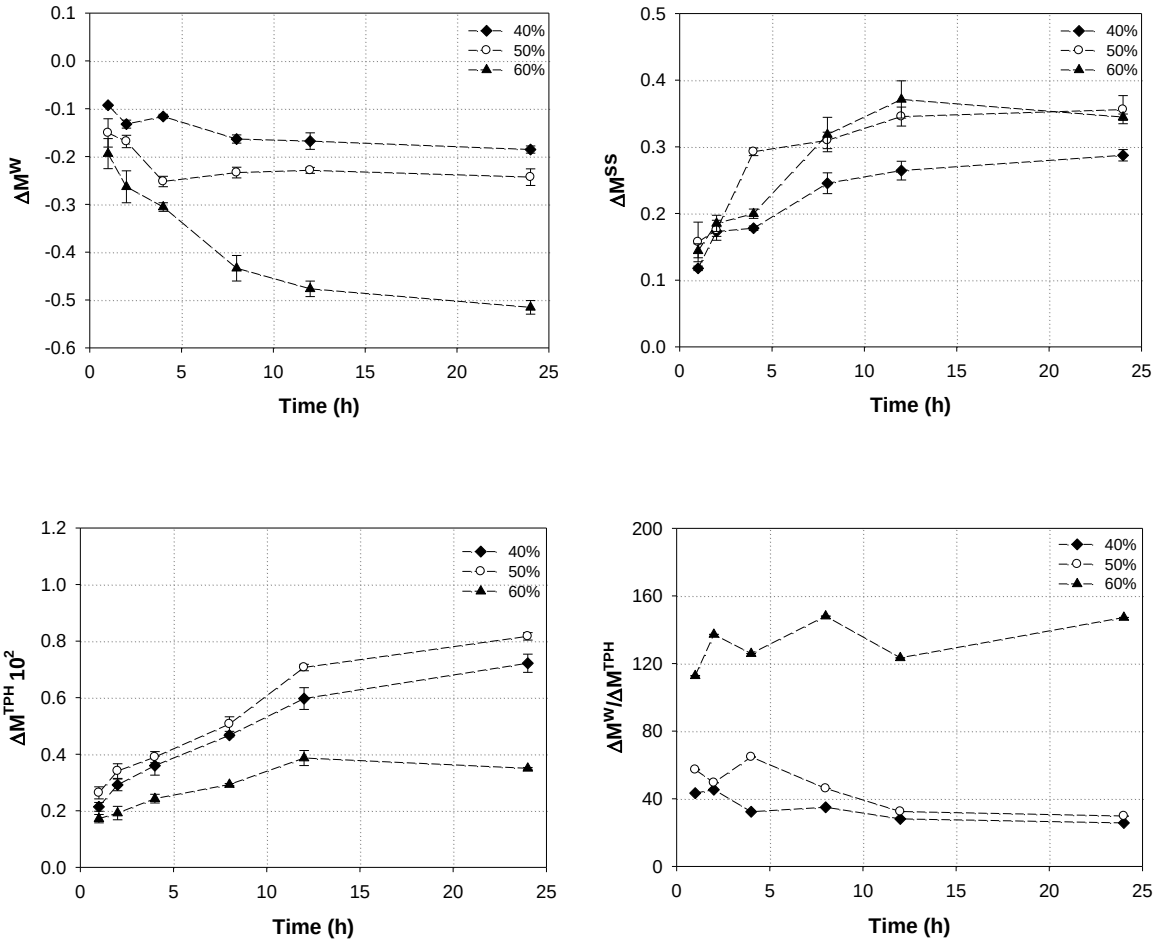

Figure 2.1. Mass changes of water (ΔM^{w}), gain in soluble solids (ΔM^{SS}) and total phenolics (ΔM^{TPH}) and ratio of water mass changes to gain in total phenolics ($\Delta M^{w}/\Delta M^{TPH}$) during OD with red grape must (mean ± standard deviation of experiments performed in triplicate). Mass fraction of soluble solids in the red must was adjusted to 40, 50, and 60%.

The highest water loss was 52% (with a 60% soluble solids mass fraction in the osmotic solution) followed by 24.3% and 18.5% (with 50 and 40% soluble solids mass fraction in the osmotic solution, respectively) for 24 h of OD. The gain in soluble solids (ΔM^{SS})

and total phenolics (ΔM^{TPH}) depends on the soluble solids concentration of the osmotic solution. While the gain of soluble solids increased with the soluble solids concentration of the osmotic solution, the total phenolic gain was highest (0.82%) with a 50% mass fraction of soluble solids in the osmotic solution. Gains of 0.72% and 0.35% were obtained, respectively, with 40 and 60% mass fractions of soluble solids in the osmotic solution. OD with 50 and 60% mass fractions of soluble solids in the osmotic solution led to a 35% gain in soluble solids, which decreased to 29% when the mass fraction of soluble solids in the osmotic solution was reduced to 40%.

Some authors (Raoult-Wack et al., 1991; Mujica-Paz et al., 2003) have reported that highly concentrated sugar solutions (mass fraction of soluble solids > 55%) hinder the penetration of the solute (solid gain) either because of a surface layer of solids, formed by the high counter current flows of water and solids, or because of the high viscosity of the solution. The slight differences between gains in soluble solids with 50 and 60% mass fractions of soluble solids in the osmotic solution support this. However, the impregnation with total phenolics (total phenolics gain) can be explained considering that total phenolics are minor components of the osmotic solution (11.13±0.28, 9.84±0.11 and 9.78±0.16 g GAE/kg in the 60, 50, and 40% soluble solid concentration of the osmotic solution, respectively) and contribute little to the osmotic pressure or a_w.

On this basis, the impregnation of total phenolics during OD with an osmotic solution of 60% mass fraction of soluble solids might be limited either by the concentrated surface layer of soluble solids or by the high viscosity of the osmotic solution, which not only prevents any further increase in soluble solids but also the penetration of other minor compounds in the osmotic solution (phenolics, for example).

Figure 2.1 depicts the progress of the ratio of water loss to phenolic gain, ($-\Delta M^{w}/\Delta M^{TPH}$). At a high concentration of soluble solids in the osmotic solution (60%), the water loss was up to 150 times greater than the phenolic gain, which indicates that dewatering is the prevailing effect with regard to impregnation. Although at lower concentrations of soluble solids in the osmotic solution (40 and 50%) dewatering was still much higher than phenolic gain water loss was between 25 and 65 times higher than phenolic gain, the ratio of water loss to phenolic gain decreased significantly.

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2.3.3. Phenolic profiles in the osmo-dehydrated food

Figure 2.2 shows the total phenolic content determined by Folin-Ciocalteu and the phenolic content determined by HPLC. The latter was calculated from the content of the individual phenolics identified and quantified in the osmo-dehydrated food and plotted in Figures 2.2 and 2.3. Hereinafter, this will be referred to as the HPLC phenolic content. The total phenolic content in the osmo-dehydrated food increased with processing time (Figure 2.2). OD for 24 h with a 50% mass fraction of soluble solids in the osmotic solution led to the highest total phenolic content in the osmo-dehydrated food (up to 7284±219 mg GAE/kg), followed by 6504±294 and 4203±106 mg GAE/kg obtained after 24h of OD with 40% and 60% soluble solids in the osmotic solution, respectively.

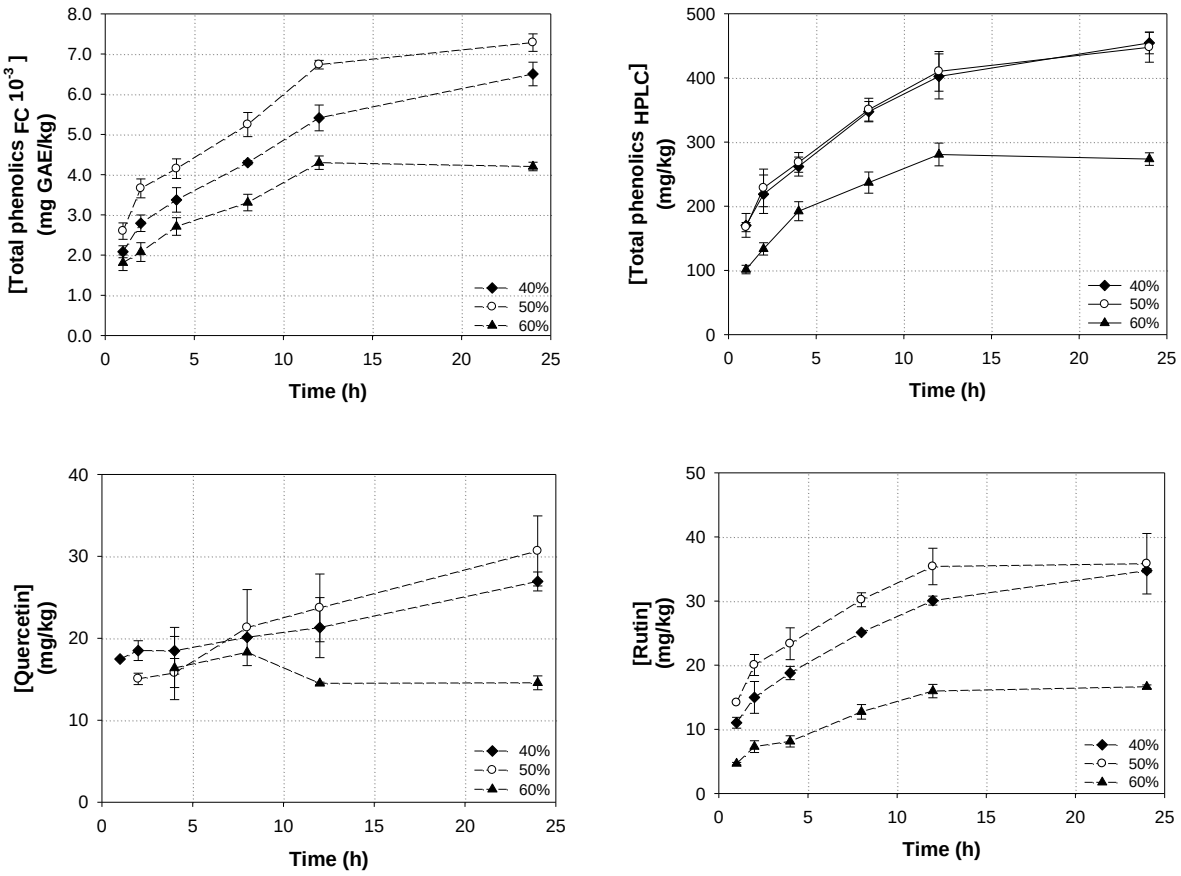

Figure 2.2. Total phenolic content, determined by Folin-Ciocalteu's method and HPLC, and content of the flavonoids (quercetin and rutin) identified in the osmo-dehydrated food during OD with red grape must (mean ± standard deviation of experiments performed in triplicate). Mass fraction of soluble solids in the red must was adjusted to 40, 50, and 60%.

Comparing these total phenolic contents with those reported in the literature for commonly consumed fresh fruit and vegetables (Chun et al., 2005; Balasundram et al., 2006; Cieslik et al., 2006) we can easily verify that the osmo-dehydrated model food obtained in these OD conditions had a similar or higher content of total phenolics than the richest fruits and vegetables. Fruits such as black and blueberries, plums, strawberries and grapefruits have a total phenolic content that may be around 267-9610, 1740-3686, 1600-2250, and 1617 mg GAE/kg FW, respectively, depending on the variety. Broccoli, cabbage or spinach, some of the vegetables that are richest in total phenolics, have a total phenolic concentration between 250-2900, 450-925, and 325-1000 mg GAE/kg FW, respectively.

Much shorter processing times, as short as 1 or 2 h of OD, would be long enough to provide osmo-dehydrated food with a total phenolic content close to that of the richest fruits and vegetables. The high concentration of total phenolics in the concentrated red must (13154 mg GAE/kg) may explain the extent of phenolic gain. Mass transfer is usually assumed to occur between the food liquid phase (i.e. food containing water and soluble components) and the osmotic solution. On this basis, the equilibrium criterion considered is that the food liquid phase and the osmotic solution are compositionally equal (Barat et al., 1998). After 24 h of OD with a 50% mass fraction of soluble solids in the osmotic solution, the phenolic content in the osmo-dehydrated food (7466 mg GAE/kg food liquid phase equivalent to 7284±219 mg GAE/kg) was still below the total phenolic content of the osmotic solution (9840±108 mg GAE/kg), which shows that equilibrium had still not been reached from the point of view of phenolic mass transfer. During OD with a mass fraction of 40, 50, and 60% of soluble solids in the osmotic solution, the concentration of individual phenolics in the osmo-dehydrated food was monitored (Figures 2.2 and 2.3). The hydroxycinnamics and gallic acid quantified in the concentrated red grape must were also detected in the osmo-dehydrated food, and were the major phenols present in the osmo-dehydrated food in all the conditions tested. However, the *cis* isomers of caftaric and coutaric acids, which were not detected in the concentrated red must, were identified in the osmo-dehydrated food. These *cis* forms are reported to be the result of a *cis*/*trans* isomerization under exposure to UV light (Betés-Saura et al., 1996). In addition, the effect of UV radiation seems to affect hydroxycinnamates differently: while in white free run juices, *trans*-coutaric was reported to be more sensitive to this isomerization than *trans*-caftaric, in the osmo-dehydrated food the opposite effect was observed.

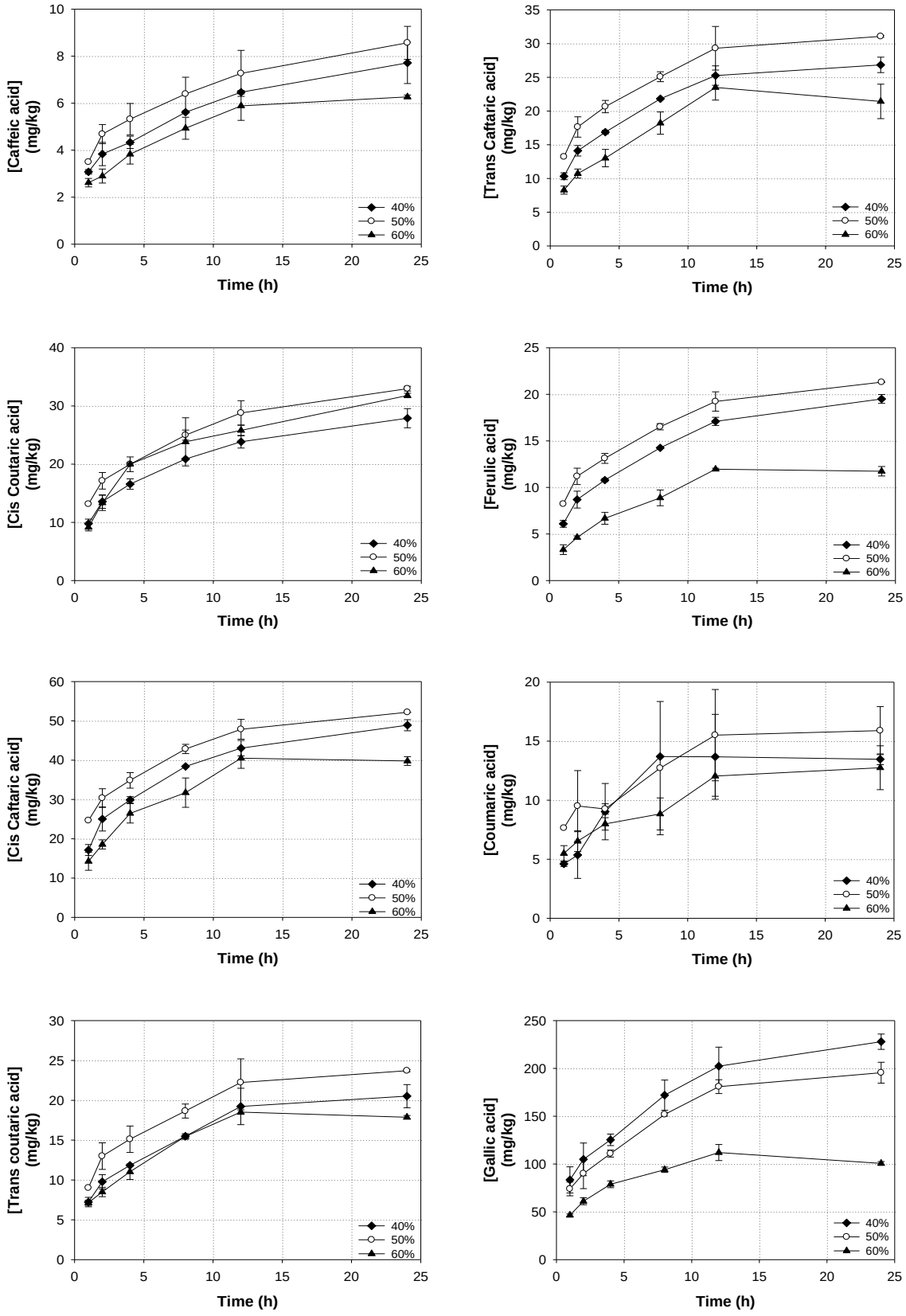

Figure 2.3. Content of the non-flavonoids (hydroxycinnamic acids and gallic acid) identified in the osmo-dehydrated food during OD with red grape must (mean ± standard deviation of experiments performed in triplicate). Mass fraction of soluble solids in the red must was adjusted to 40, 50, and 60%.

The ratio of *cis*-coutaric to *trans*-coutaric concentration was slightly lower than that of *cis*-caftaric to *trans*-caftaric concentration in all the cases analyzed (Figure 2.3).

Of the phenolics identified and quantified in the concentrated red must (Table 2.1), the only flavonoids that were detected in the osmo-dehydrated food in all the operating conditions considered were quercetin and rutin. Other flavonoids such as (+)-catechin and (–)-epicatechin were not detected in any of the samples of the osmo-dehydrated food analyzed. As the molecular weight of these compounds is in the same range as the others detected in the osmo-dehydrated food, (+)-catechin and (–)-epicatechin might be absent because they were oxidized during OD or further extraction steps. The high antioxidant potential of both compounds has been extensively reported (Rice-Evans et al., 1996) and although the pH of the grape must was maintained at 3.4 in order to prevent the phenolics from oxidizing, agitation during OD and contact with air during the further extraction might oxidize them both.

The influence of the mass fraction of soluble solids of the osmotic solution on the gain in individual phenolics was the same as on the gain in total phenolics. The phenolic concentration in the osmo-dehydrated food was highest in all the cases for the 24 h of OD with a 50% mass fraction of soluble solids in the osmotic solution. In these conditions, gallic acid was the phenolic found in the highest concentration (227.9 mg/kg) (Figure 2.3). Of the hydroxycinnamics (Figure 2.3), *cis*-caftaric was found at the highest level (52.2 mg/kg), followed by *cis*-coutaric acid (33.0 mg/kg). Both *trans* isomers of caftaric and coutaric acids were detected in lower concentrations (31.1 mg/kg and 23.7 mg/kg, respectively). Ferulic acid (21.3 mg/kg) was the free cinnamic acid found at the highest concentration, followed by coumaric (15.9 mg/kg) and caffeic acids (8.6 mg/kg).

In the case of flavonoids, the concentration of quercetin and rutin in the osmo-dehydrated food after 24 h of OD with an osmotic solution of 50% mass fraction of soluble solids was 30.7 and 35.8 mg/kg, respectively.

According to these results, OD proved to be an efficient operation for supplementing a solid foodstuff with grape phenolics when a concentrated red grape must was used. Adjusting the operating conditions (basically the soluble solids content in the osmotic solution) makes it possible to maximize phenolic impregnation. However, other operating conditions such as the total phenolic content of the grape must and agitation/aeration should be optimized. In particular, the effect of agitation/aeration on

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the oxidation of such phenolics as (+)-catechin and (–)-epicatechin, should be further investigated. The application of OD to supplement real foods requires additional research in order to determine the influence of the food structure and composition on the impregnation pattern of phenolics.

2.3.4. Antioxidant capacity of the osmo-dehydrated food

A one-assay protocol cannot evaluate the effectiveness of antioxidants in complex heterogeneous foods since antioxidant protection involves several mechanisms. Two in-vitro antioxidant capacity assays, TEAC and FRAP, were chosen to measure the free radical scavenging activity and the total reducing power, respectively, in the osmo-dehydrated food during OD. Although these assays are non-specific and provide little information about the mechanisms controlling the antioxidant action, they are both widely used to determine antioxidant capacity in foods and they have provided a great deal of antioxidant data (Frankel et al., 2000).

Figure 2.4 shows the increase in TEAC and FRAP during OD with a 40, 50, and 60% mass fraction of soluble solids in the osmotic solution. As observed with total and individual phenolics, the antioxidant capacity measured by both methods was highest with a soluble solid mass fraction of 50% in the osmotic solution. In these conditions and after 24 h of OD, TEAC and FRAP values were 66.3 and 89.9 mmol Trolox/kg, respectively. In general, berries are the edible fruits that have the greatest antioxidant capacity. Reported TEAC values of blackberry, raspberry and strawberry are 20.24, 16.79, and 10.94 mmol Trolox/kg FW, respectively (Pellegrini et al., 2003).

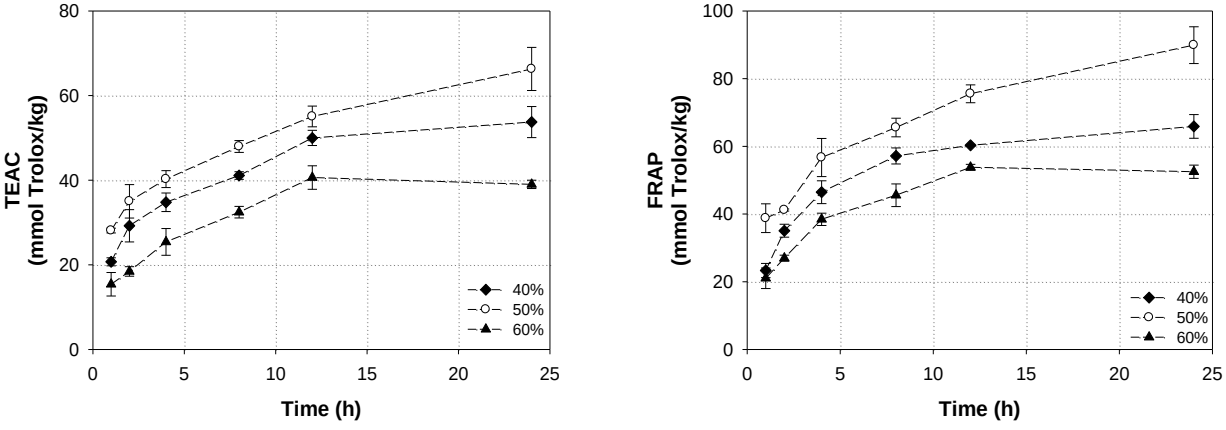

Figure 2.4. Antioxidant activity of the osmo-dehydrated food measured as TEAC and FRAP during OD with red grape must.

Vegetables usually have lower antioxidant capacity than fruits, and spinach and peppers have been found to have the highest (TEAC values of 8.49 and 8.40 mmol Trolox/kg FW, respectively). So, with OD in the conditions that led to the highest phenolic gain (50% mass fraction of soluble solids in the osmotic solution and 24 h) it was possible to obtain an end product with a TEAC value that was three times higher than that observed in the fruits with highest antioxidant capacity. At shorter processing times, between 2 and 4 h, and with any of the three osmotic solutions used (40, 50, and 60% mass fraction of soluble solids), TEAC values ranged from 20 to 40 mmol Trolox/kg: that is, between 1 and 2 times the antioxidant capacity of the berries.

Comparing these results with the total gain in phenolics, we observed that under the same operating conditions (50% mass fraction of soluble solids in the osmotic solution and 24 h), the total phenolic content was at the same level as the fruit that was richest in polyphenols while the antioxidant capacity, in terms of TEAC, was 3 times the antioxidant capacity of the most antioxidant fresh fruits.

Data from TEAC and FRAP correlated well: the effect of OD conditions on both parameters was comparable. On average, FRAP values were 31% higher than the TEAC values (FRAP = 1.31×TEAC + 0.69, r^2 = 0.966). These differences, previously reported by other authors (Saura-Calitxo et al., 2006; Nilsson et al., 2005), have been related to the fundamental characteristics of both methods. TEAC shows the ability of an antioxidant to scavenge the artificial $ABTS^{\cdot+}$ radical while FRAP measures its reducing capacity. However, neither of them reflects the antioxidant capacity due to other effective mechanisms. As the main goal of this study is to assess whether OD with a concentrated red must, in the operating conditions considered, can increase the antioxidant capacity of a model food (gel in this case) to the same or higher levels than those of the most antioxidant fresh fruits, both FRAP and TEAC values are used to complement the phenolic profile.

2.3.5. Correlations between phenolic content and antioxidant capacity

Correlations between the antioxidant capacity and phenolic profile were determined in order to detect the extent to which the phenolics identified by HPLC describe the antioxidant capacity of the osmo-dehydrated food. Regression analysis (Table 2.2) showed that the HPLC phenolic content was significantly correlated with TEAC (r^2 = 0.936, P<0.001) and FRAP (r^2 = 0.873, P<0.001) but the correlation between the antioxidant capacity and total phenolic content, determined with the Folin-Ciocalteau

method, was slightly higher: TEAC (r^2 = 0.971, P<0.001) and FRAP (r^2 = 0.936, P<0.001). Considering that the total phenolic content determined by Folin-Ciocalteau in the osmo-dehydrated food was approximately one order of magnitude higher than that determined by HPLC (Figure 2.2), the regression analysis suggests that other phenolics that are present in the osmo-dehydrated food have a rather low impact on the free radical scavenging activity and the total reducing power.

Table 2.2. Linear regression analysis of antioxidant capacity, TEAC and FRAP, versus individual and total phenolics content

	TEAC				**FRAP**			
Phenolics	**a ***	**b ***	**r^2**	**P ****	**a ***	**b ***	**r^2**	**P ****
caffeic acid	7.72	-2.55	0.956	$2.61\ 10^{-12}$	10.14	-2.78	0.928	$1.42\ 10^{-10}$
cis-caftaric acid	1.18	-2.30	0.951	$7.18\ 10^{-12}$	1.56	-2.80	0.935	$6.63\ 10^{-11}$
trans-caftaric acid	1.98	-0.77	0.967	$2.72\ 10^{-13}$	2.61	-0.74	0.949	$8.58\ 10^{-12}$
coumaric acid	3.53	1.36	0.856	$3.88\ 10^{-08}$	4.74	1.22	0.871	$1.61\ 10^{-08}$
cis-coutaric acid	1.61	4.03	0.735	$5.48\ 10^{-06}$	2.20	4.05	0.773	$1.54\ 10^{-06}$
trans-coutaric a.	2.49	0.86	0.912	$7.38\ 10^{-10}$	3.31	1.04	0.909	$9.46\ 10^{-10}$
ferulic acid	2.55	7.21	0.979	$8.95\ 10^{-15}$	3.30	10.55	0.925	$2.07\ 10^{-10}$
gallic acid	0.23	8.93	0.809	$3.81\ 10^{-07}$	0.29	13.89	0.719	$8.71\ 10^{-06}$
quercetin	2.05	0.77	0.617	$5.20\ 10^{-04}$	2.68	1.50	0.558	$1.37\ 10^{-03}$
rutin	1.30	11.48	0.909	$9.57\ 10^{-10}$	1.67	16.41	0.843	$7.98\ 10^{-08}$
TPH_{FC}[a]	$8.13\ 10^{-03}$	4.65	0.971	$1.12\ 10^{-13}$	$1.06\ 10^{-02}$	6.81	0.936	$5.91\ 10^{-11}$
$hydroxycinnamics_{HPLC}$	0.33	-0.72	0.946	$1.50\ 10^{-11}$	0.438	-0.955	0.939	$3.91\ 10^{-11}$
$flavonols_{HPLC}$	0.73	11.02	0.879	$9.85\ 10^{-09}$	0.929	16.02	0.805	$4.56\ 10^{-07}$
TPH_{HPLC}[b]	0.124	3.42	0.936	$5.69\ 10^{-11}$	0.159	5.94	0.873	$1.46\ 10^{-08}$

a. b Total phenolics determined using the Folin-Ciocalteau method and HPLC, respectively.
* **a** (mmol Trolox/mg of phenol) and **b** (mmol Trolox/kg) are the slope and the intercept.
**** P** - values of the regression coefficients.

When TEAC and FRAP were correlated with the total contents of the various phenolic groups, statistically significant dependencies could be stated. The total content of hydroxycinnamics, hydroxybenzoic acids and flavonols identified by HPLC was highly correlated with both TEAC and FRAP. The highest correlation was shown by hydroxycinnamics (TEAC: r^2 = 0.946, P<0.001, and FRAP: r^2 = 0.939, P<0.001), followed by flavonols (TEAC: r^2 = 0.879, P<0.001, and FRAP: r^2 = 0.805, P<0.001), and gallic acid (TEAC: r^2 = 0.809, P<0.001, and FRAP: r^2 = 0.719 P<0.001).

In the hydroxycinnamics group, all the phenolics analyzed exhibited a high and significant correlation with TEAC and FRAP. Ferulic acid and caffeic acid (and both isomers of its tartrate ester, *cis*-caftaric and *trans*-caftaric acids), however, showed the highest correlation, with r^2 values above 0.95 and 0.92 for TEAC and FRAP, respectively. Coumaric acid and its tartrate esters, *cis*-couratic and *trans*-coutaric acids,

exhibited the weakest correlations in the hydroxycinnamics group although they were still significant (r^2 values between 0.735 and 0.909). Gallic acid, the only hydroxybenzoic acid analyzed, was significantly correlated with TEAC and FRAP, but its correlation was low compared to its high contents in the osmo-dehydrated food (up to 4.5 times that of the major hydroxycinnamics). The correlations of the individual flavonols analyzed with TEAC and FRAP were significant ($P<0.001$) and higher for rutin than for quercetin. This can only be explained by taking into account the high dispersion obtained in the quercetin determinations.

These results showed that the individual phenolics analyzed significantly explain the antioxidant capacity of the osmo-dehydrated food in terms of free radical scavenging activity and the total reducing power. However, the statistical analysis used, a simple linear regression, did not seem able to properly evaluate the contribution of each individual phenolic to the antioxidant capacity. What can be inferred from the above correlations may, in some cases, not fit with what has been reported (Rice-Evans et al., 1996). A further statistical analysis should be designed so that the possible synergic effects that might mask the present results can be evaluated.

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CHAPTER 3

*MASS TRANSFER RATE OF LOW MOLECULAR PHENOLICS DURING OSMOTIC TREATMENT WITH RED GRAPE MUST**

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3.1. INTRODUCTION

In recent years, industrial and consumer interests have focused on developing foods supplemented with physiologically active components that provide greater physiological benefits (Mazza et al., 1998). The direct addition of these components to liquid or fabricated solid foods has led to a wide range of new products appearing on the market.

Osmotic dehydration (OD), an operation in which a foodstuff is soaked in a solution of low water activity, has been reported to be suitable for formulating new products because of the twofold effect that it has on food: it partially removes water and impregnates the food piece with solutes from the osmotic solution (Barbosa-Cánovas et al., 1995; Shi et al., 1996; Nsozi et al., 1998; Spiess et al., 1998; Bonazzi et al., 1996). By controlling the operating conditions (time, pressure, temperature, nature of the raw material, kind and concentration of the osmotic solute, etc.) the ratio of water loss to solute impregnation can be adjusted (Bonazzi et al., 1996; Hui Cao et al., 2006; Pan et al., 2003). OD at low pressure (i.e. vacuum impregnation) has been applied to the Ca and Fe fortification of fruit (apple) and vegetables (Barrera et al., 2004). It has been reported that the choice of osmotic solute controls the ratio of water loss to solute impregnation. Sugars (for fruits) and inorganic salts (for vegetables, fish, meat, and cheese) are the most common solutes used in OD and the combination of both kinds of solutes, in ternary sucrose/salt/water solutions, can reduce solute impregnation and increase water loss (Sereno et al., 2001). Fruit juices have been used as an osmotic solution (Escriche et al., 2000) although little attention has been paid to the infusion of minor solutes from these multicomponent osmotic solutions. Grape must is a water solution (76 %, w/w) made of sugars (23 %, w/w, mainly glucose and fructose followed by sucrose), organic acids (0.7 %, w/w), inorganic salts (0.4 %, w/w), nitrogen compounds (0.1 %, w/w), lipids (0.01 %, w/w), and also phenolics (0.01 %, w/w) (Boulton et al., 1995). Phenolics in grape must are a complex mixture of compounds that includes non-flavonoids (hydroxycinnamates, hydroxybenzoates and stilbenes), flavonoids (flavan-3-ols), flavonols, and anthocyanins. Despite their relatively low concentration in comparison to other grape compounds, their role in human health makes grape phenolics one of the main goals of food companies. Because of their properties as natural antioxidants, grape phenolics prevent such pathological disorders as cardiovascular diseases and cancer (Yilmaz et al., 2004).

By-products of the food industry, particularly the waste of fruit and vegetable processing are rich in phenolics. The wine-making industry produces a huge amount of these residues (that is seeds, skins and highly pressed must) from which extracts with a high phenolic content are obtained. Their application to the development of food products of high nutritional value has so far been limited to supplement confections, fruit fillings, sauces, beverages and pasta products but no application has been reported for infusing fresh solid foodstuffs.

To evaluate the possibilities that OD can be used to develop products that are not only impregnated with a major osmotic solute but also with a controlled and significant amount of target solutes of high nutritional value, the mass transfer rates of water and several solutes need to be described.

To describe mass transfer during OD, two basic approaches (i.e. macroscopic and microscopic) have been considered (Le Maguer et al., 1995). Most of the models start from a macroscopic approach, which applies Fick's second law for unsteady state diffusion to model water and solute transport between the osmotic solution and the food piece (Azuara et al., 1992; Beristain et al., 1990; Hough et al., 1993; Lazarides et al., 1997; Rastogi et al., 2002; Simal et al., 1998). The microscopic approach takes the heterogeneous properties of the foodstuff into account since it was developed to model mass transfer in such very structured and compartmental systems as plant tissue. These models use an extended form of Fick's second law for the unsteady state, incorporating transmembrane flux and shrinkage of the matrix (Marcotte et al., 1991; Spiazzi et al., 1997). In the macroscopic or diffusional approach, effective diffusion coefficients, calculated by regressing experimental data, are phenomenological coefficients that are strongly affected by interactions between water and solute flows, microstructural effects, and changes in the physical properties of the food as a result of OD (Raoult-Wack et al., 1994). In spite of this, the approach is still widely used because of the considerable amount of existing data, the straightforward way in which diffusion coefficients are calculated and the fact that the physical properties of the food material need not be known.

The aim of this study was to investigate the mass transfer of minor solutes in a multicomponent solution during OD. On the basis of the analytical solution to Fick's second law, we evaluated the effective diffusion coefficients of water and solutes, including total phenolics and some individual phenolics of low molecular weight, during the OD of a model food (an agar-agar gel) with a concentrated red grape must.

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3.2. MATERIALS AND METHODS

3.2.1. Preparation of model food and osmotic solution

The model food, an agar-agar gel, was prepared with agar-agar (Scharlau, Spain), sucrose and distilled water with 4.0%, 9.6% and 86.4% (w/w), respectively. The mixture was heated up to 95°C in a microwave oven until the agar-agar was completely dissolved. It was then gelated by cooling to room temperature. Prior to use, the gel was stored at 6° ± 2°C. Concentrated red grape must (vars. *Bobal*, *Garnacha* and *Tintorera*) was supplied by Concentrados Palleja, S.L. Red grape must (65° Brix and pH 3.5) was diluted in water to 40, 50 and 60° Brix. Tartaric acid (1 g/L) was added to prevent pH changes.

3.2.2. Osmotic dehydration

The experimental set-up consisted of two parts: a basket in which the gel samples were placed and a vessel that was filled with the osmotic solution. The basket contained five shelves and guaranteed that the sample could be totally immersed in the osmotic solution. The solution was agitated by a magnetic stirrer. The agitation level was chosen in order to make the surface mass transfer negligible. About 150g of agar-agar gel cubes were weighed and placed in the OD basket. The basket was then submerged in 2.7 L of osmotic solution. The model gel was processed for 1, 2, 4, 8, 12 and 24 hours and the osmotic pressure was adjusted by diluting the concentrated red grape must to 40, 50 and 60° Brix. A 20:1 ratio (w/w) of solution: gel prevented changes in the solution concentration. During the experiment, the temperature was maintained at 25º ± 2ºC and the set-up was covered to minimize the effect of light. After osmotic dehydration, the gel cubes were removed from the solution, their surface was gently blotted with a tissue paper and they were weighed. Each experiment was carried out in triplicate. All experiments were run under atmospheric pressure.

3.2.3. Determination of moisture and soluble solids content

The moisture content of fresh and osmotically treated model food was determined following the 934.06 AOAC method (1998). The concentration of soluble solids in osmotic solutions was determined using a refractometer (932.14 AOAC, 1998).

3.2.4. Extraction of phenolics

To determine the extent of phenolic impregnation in the gel after osmotic dehydration, a sequential extraction was carried out. A total of 2.5g of grinded sample was extracted sequentially with 15 mL of methanol: water (50/50, v/v) and 15 mL of acetone: water (50/50, v/v), for 1 hour in each extraction solvent at room temperature. Each solvent extraction was carried out in triplicate.

3.2.5. Determination of total phenolic content

The total phenolic content of red grape must and gel extracts was determined with the Folin-Ciocalteu method (Singleton et al., 1965). The test sample (1 mL) was mixed with 50 mL of distilled water, 5 mL of Folin-Ciocalteu's reagent and 20 mL of 20% sodium carbonate solution. After 30 min, the absorbance at 750 nm was recorded. The results were expressed as gallic acid equivalents (mg GAE/g gel).

3.2.6. Identification and quantification of individual phenolics by HPLC

Polyphenols were identified by HPLC (Hewlett-Packard (HP)/Agilent). Injection was by means of an automatic injector, HP 1000. A Supelcosil^{TM} LC-18 column (25 cm × 4.6 mm), with a particle size of 5 μm and an injection volume of 100 μl was kept at 40°C. A constant flow rate of 1.5 mL/min was used with two solvents: solvent A (acetic acid in water at a pH of 2.60) and solvent B (20% solvent A mixed with 80% acetonitrile). Peaks monitored by an HPLC system equipped with a diode-array detector were identified by their retention times and spectra in comparison with external standards. A diode array UV-vis detector (DAD) made it possible to choose the maximum absorbance for each group of compounds, control the peak purity, and identify the spectra of some phenols. Concentrations of identified phenolic compounds were measured using external standard curves. Calibration curves (standard area in absorbance versus concentration in mg/L) were performed over the range of concentration observed. Trans caftaric acid, trans-coutaric acid, caffeic acid, coumaric acid, ferulic acid, and rutin, were purchased from Sigma-Aldrich (Germany).

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3.2.7. Determination of effective diffusion coefficients of water and osmotic solutes

The effective diffusion coefficients for water and osmotic solutes were calculated to evaluate OD. The analytical solution of Fick's second law for cubical configuration was used to model the mass transfer of water and osmotic solutes during OD (Crank et al., 1975), assuming that model food cubes behave like isotropic structures. The initial and boundary conditions assumed that the cube's initial content of water and soluble solids was uniform, that the food solid was symmetrical with regard to the mass transfer direction, and that the system was in thermodynamic equilibrium. Mass transfer was also assumed to occur between the food liquid phase (i.e. food containing water and soluble components) and the osmotic solution. On this basis, the equilibrium criterion considered was that the food liquid phase and the osmotic solution were compositionally equal (Barat et al., 1998; Barat et al., 1999). With all these assumptions:

$$M_r = \frac{\left(z_t^w - z_\infty^w\right)}{\left(z_0^w - z_\infty^w\right)} = \left\{\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-(2n+1)^2\frac{\pi^2 D_{ew}}{4a^2}t\right]\right\}^3 \quad (3.1)$$

$$TPH_r = \frac{\left(z_t^{TPH} - z_\infty^{TPH}\right)}{\left(z_0^{TPH} - z_\infty^{TPH}\right)} = \left\{\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-(2n+1)^2\frac{\pi^2 D_{eTPH}}{4a^2}t\right]\right\}^3 \quad (3.2)$$

$$PH_{jr} = \frac{\left(z_t^{PHj} - z_\infty^{PHj}\right)}{\left(z_0^{PHj} - z_\infty^{PHj}\right)} = \left\{\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-(2n+1)^2\frac{\pi^2 D_{ePHj}}{4a^2}t\right]\right\}^3 \quad (3.3)$$

where M_r, TPH_r, and PH_{jr} are the moisture, and the total phenolic and the phenolic ratio of an individual phenolic *j*; *z* is the mass fraction in the food liquid phase; the subscripts 0,∞, and t represent initial concentrations, at equilibrium, and at any time; the superscripts *w*, *TPH*, and *j* represent water, total phenolics, and an individual phenolic; D_e is the effective diffusion coefficient, and *a* is the half thickness of the cube.

Values of z were calculated as:

$$z_t^i = \frac{x_t^i}{\left(x_t^w + x_t^{ss} + x_t^{TPH}\right)} \quad (3.4)$$

where *x* is the mass fraction in the food of the component i. As the refractometer's method provided information about the soluble solid content in the food liquid phase, z_t^{ss}, the mass fraction of soluble solids in the food was calculated as:

$$x_t^{ss} = \frac{z_t^{ss}(x_t^w + x_t^{TPH})}{\left(1 - z_t^{ss}\right)} \tag{3.5}$$

The values of D_e were determined by regressing experimental data to equations 1-3. The fitting was performed with SigmaPlot version 9.01 by applying the Marquardt-Levenberg algorithm. This algorithm searches for the values of the parameter D_e that minimize the sum of the squared differences between the values of the observed and predicted values of the dependent variable. The regression coefficient, r, was used to measure how well the regression model described the data.

3.3. RESULTS AND DISSCUSION

3.3.1. Characterization of the multicomponent solution: Phenolic profile

In this study, the phenolics that were identified and quantified were chosen because of their high concentration in grape must, their antioxidant capacity and their low molecular weight. Table 3.1 lists the phenolics identified and quantified and shows their molecular structure and classification. All the phenolics in Table 3.1 are easily oxidized and have a greater or lesser degree of antioxidant activity. The molecular weight of grape phenolics can range from 150 to several thousand g/mol since some of them are the result of condensation reactions. The molecular weight of the phenolics that have been identified and quantified in this study ranges between 164 and 610 g/mol, which is of the same order of magnitude as the main sugars present in grape must (glucose, fructose and sucrose), whose molecular weights are between 180 g/mol and 342 g/mol. The soluble solids concentration of the red must concentrate, at 65º Brix, was approximately three times that of a standard grape must.

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Table 3.1. Properties of the individual phenolics identified in the red must and model food after OD

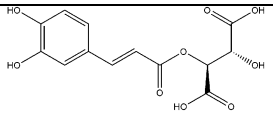
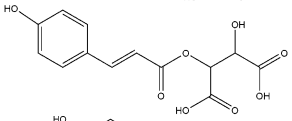
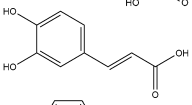
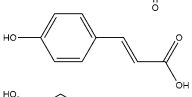
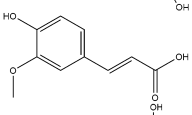
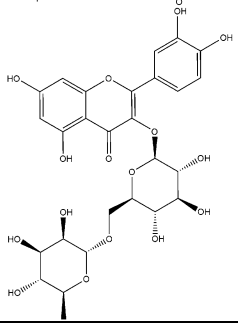
	Molecular weight [g/mol]	Molecular structure	Phenolic classification
Caftaric acid	312.23		Non-flavonoids: Hydroxycinnamates
Coutaric acid	295.00		
Caffeic acid	180.20		
Coumaric acid	164.16		Non-flavonoids: Hydroxycinnamic acids
Ferulic acid	194.18		
Rutin	610.53		Flavonoids: Flavonol

Table 3.2 shows the soluble solids, and total phenolic and individual phenolic content of the red grape must at the several dilutions used during OD. Although the individual phenolics identified belong to major groups, it should be pointed out that they are just a few of the numerous phenolics that a red grape must can have: approximately 3% (w/w) of the total phenolics (Table 3.2).

Table 3.2. Composition of the red grape must at the various concentrations used during OD. Mean and standard error are presented

Experiment Labeling	60º Brix	50º Brix	40º Brix
Soluble Solids [ºBrix]	59.4±1.38	53.0±2.60	41.0±2.55
Total phenolics [g GAE/kg]	11.134±0.276	9.840±0.108	9.784±0.160
Caftaric acid [g/kg]	0.134±0.004	0.115±0.006	0.101±0.002
Coutaric acid [g/kg]	0.094±0.006	0.081±0.004	0.071±0.003
Caffeic acid [g/kg]	0.011±0.002	0.009±0.0005	0.008±0.0004
Coumaric acid [g/kg]	0.035±0.002	0.022±0.002	0.021±0.002
Ferulic acid [g/kg]	0.030±0.002	0.030±0.003	0.024±0.001
Rutin [g/kg]	0.071±0.001	0.056±0.003	0.056±0.002
Individual phenolics [g/kg]	0.376±0.015	0.313±0.012	0.280±0.006
Individual phenolics/ Total phenolics [%]	3.400±0.084	3.200±0.035	2.900±0.046

The data in Table 3.2 show that the predominant hydroxycinnamate in grape must was caftaric acid (0.101 to 0.134 g/kg), followed by coutaric acid (0.071 to 0.094 g/kg). In the hydroxycinnamic acids, coumaric acid was found at the highest concentration followed by ferulic and caffeic acids. This phenolic profile is slightly different to that of a fresh grape must in which caftaric acid is also predominant but at concentrations averaging 0.170 g/kg. Coutaric acid occurs at about 0.020 g/kg and the simple hydroxycinnamic acids are not found (Barat et al., 1999). The methods used to concentrate the red must are probably the reason for the partial hydrolysis and oxidation of some these compounds. All the phenolics listed in Table 3.1 were detected at significant amounts in the food samples after OD in all the conditions considered.

3.3.2. Mass transfer during OD: Effect of the soluble solids concentration in the osmotic solution

To characterize and compare mass transfer during OD in different operating conditions, the changes in the moisture and total phenolics in the model food were calculated as dimensionless values related to the initial and equilibrium conditions. Figure 3.1 plots the moisture and total phenolic ratio against processing time during OD with a red grape must of 40, 50 and 60º Brix.

a) b)

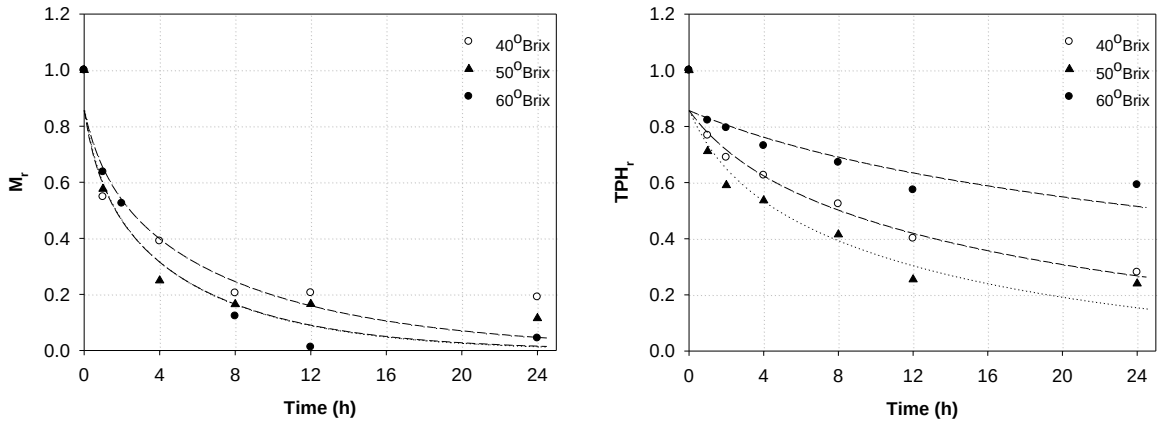


Figure 3.1. Moisture (a) and total phenolics (b) ratio during OD with 40, 50 and 60º Brix red must. Lines are the fitted model.

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In some conditions, the values of moisture ratio at 24 h did not approach zero. These unexpected results have been associated to experimental error; the mass fraction of the fluid liquid phase was calculated by equations (3.4) and (3.5) in which not only moisture determinations but also soluble solids (by refractometer method) and total phenolic analysis (by Folin-Ciocalteu method) were involved.

Figure 3.1 shows that an increase in the soluble solids concentration of the must increased the rate of water transport, as has been widely reported in several kinds of foodstuffs (Spiess et al., 1998). In our case, the high ratio of soluble solids to phenolic mass in the must (about 40 for all the conditions) suggests that water transport was mainly controlled by the soluble solids content, acting as osmo-active solutes.

As far as the mass transfer of total phenolics is concerned, the rate of impregnation was also controlled by the soluble solids concentration of the osmotic solution. The process was fastest with the 50º Brix solution, but increasing the concentration to 60º Brix, significantly reduced the mass transfer rate. During OD with sucrose solutions, this behavior for sucrose mass transfer has already been reported in agar-agar gels (Raoult-Wack et al., 1991) and in some plant foods like apple treated under vacuum (Mujica-Paz et al., 2003). In the case of agar gels treated by OD under atmospheric pressure, this effect has been explained by the cross-flows of solutes and water: at high solute concentrations (>50º Brix of a sucrose solution) the high water flow at the beginning of the OD concentrates the osmotic solute on the food surface, hindering further solute impregnation. During OD under vacuum pressure, the viscosity of the osmotic solution seems to control the solute penetration: osmotic solutions of low viscosity (<50º Brix of a sucrose solution) massively penetrate the food while osmotic solutions of high viscosity (>55º Brix of a sucrose solution) have difficulty in penetrating.

The progress of the individual phenolics during processing is plotted in Figures 3.2 and 3.3. The effect of the soluble solids concentration on the mass transfer rate is similar for all the individual phenolics analyzed. As pointed out for total phenolics, the ratio of each individual phenolic significantly decreased during OD with 60º Brix red must while OD with 40 and 50º Brix red must led to the highest impregnation rates. By using the results shown in Figures 3.1, 3.2, and 3.3 and the proposed model (equations 1- 3), we calculated the effective diffusion coefficients of moisture, total phenolics, and the six phenolics identified.

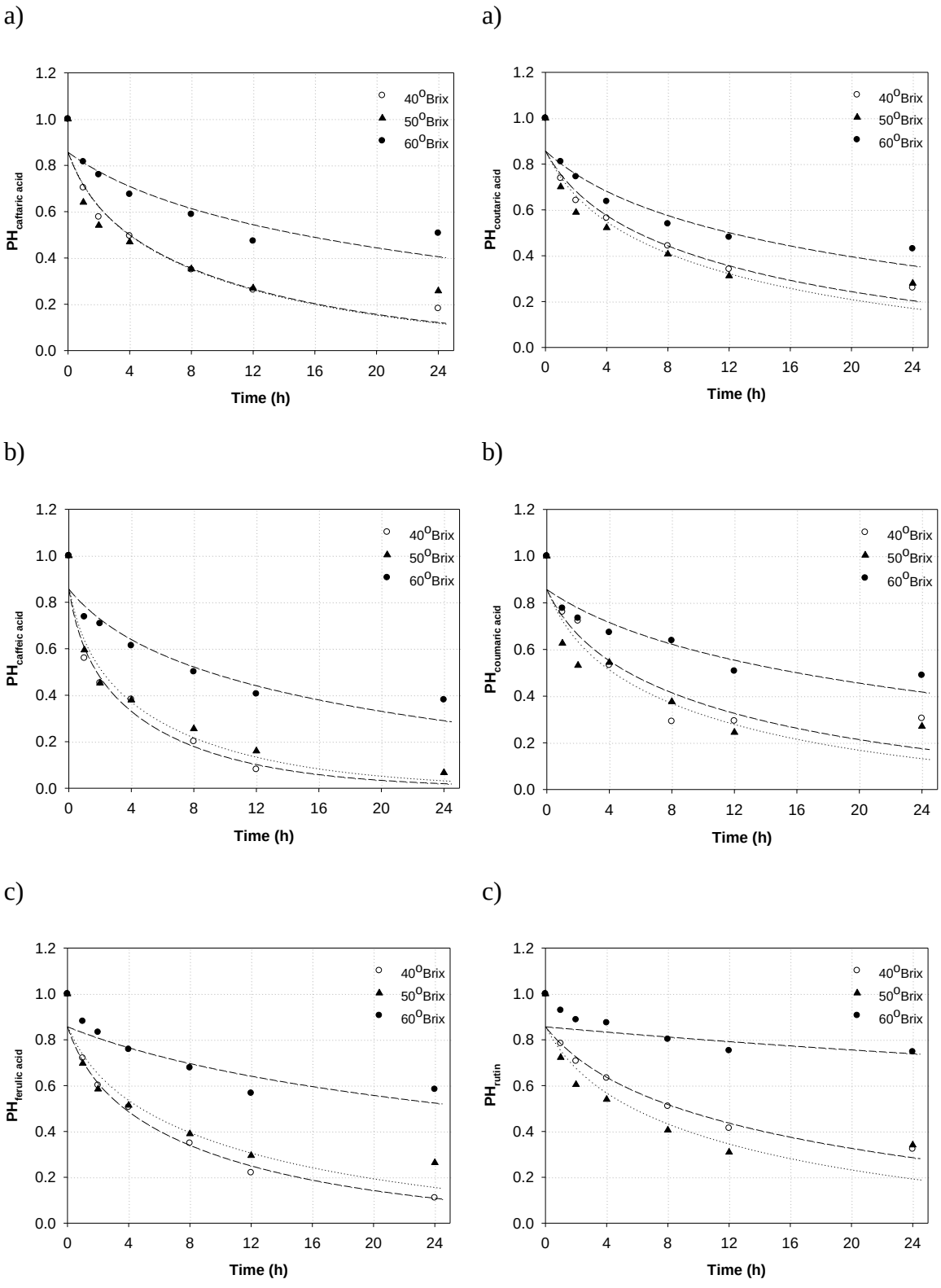

Figure 3.2. Ratio of caftaric acid (a), caffeic acid (b), and ferulic acid (c) during OD with 40, 50 and 60º Brix red must. Lines are the fitted model.

Figure 3.3. Ratio of coutaric acid (a), coumaric acid (b), and rutin (c) during OD with 40, 50 and 60º Brix red must. Lines are the fitted model.

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Table 3.3 summarizes these effective diffusion coefficients for all the operating conditions studied. Values for moisture D_e ranged from 9.5 10^{-11} to 13.9 10^{-11} m^2/s with red grape must of 40º and 60º Brix, respectively. Since the Fickian approach has been used very little to model water transport during the OD of gels, no data on moisture effective diffusion coefficients could be found.

Table 3.3. Effective diffusion coefficients of moisture, total phenolics and individual phenolics during OD with red grape must of 40º, 50º and 60º Brix

	40º Brix		50º Brix		60º Brix	
	D_e x 10^{11} [m^2/s]	R^2	D_e x 10^{11} [m^2/s]	R^2	D_e x 10^{11} [m^2/s]	R^2
Water	9.53 ±2.46	0.888	13.9±3.27	0.932	13.9±3.12	0.956
Total phenolics	2.88±0.24	0.935	4.86±0.83	0.919	0.90±0.02	0.760
Caftaric acid	5.82±0.85	0.944	5.86±1.21	0.871	1.54±0.35	0.818
Coutaric acid	3.82±0.58	0.932	4.48±0.82	0.894	1.93±0.36	0.878
Caffeic acid	13.0±2.2	0.948	10.8±1.69	0.951	2.60±0.48	0.881
Coumaric acid	4.42±0.98	0.877	5.43±1.21	0.848	1.45±0.31	0.817
Ferulic acid	6.24±0.84	0.960	4.81±0.86	0.904	0.86±0.22	0.782
Rutin	2.65±0.40	0.929	4.02±0.85	0.857	n.s. *	-

***** n.s.- not significant because P>0.05.

The D_e of total phenolics was lower than the moisture D_e for all the conditions tested and was significantly influenced by the soluble solids concentration of the red must. D_e increased from 2.9 10^{-11} to 4.9 10^{-11} m^2/s during OD with 40 and 50º Brix red must and significantly decreased to 0.9 10^{-11} m^2/s during OD with 60º Brix red must. Values of D_e for individual phenolics ranged from 0.86 10^{-11} to 13.9 10^{-11} m^2/s depending on the kind of phenolics and the soluble solids concentration of the red must. Like the D_e of total phenolics, the D_e of all the individual phenolics decreased considerably during OD with 60º Brix. OD with 40 and 50º Brix did not lead to significant differences in the D_e of hydroxycinnamates (caftaric and coutaric acid) or hydroxycinnamic acids (caffeic, coumaric and ferulic). However, the D_e of the only flavonoid analyzed (rutin) significantly increased from 2.65 10^{-11} to 4.02 10^{-11} m^2/s during OD with 40 and 50º Brix respectively.

Of all the individual phenolics analyzed, rutin showed the lowest values of D_e in all the conditions studied. The molecular weight of rutin, 610 g/mol, which is 2-4 times greater than the molecular weight of the other phenolics analyzed, may explain this result. It has been widely reported that molecular weight of the osmotic agent affects impregnation: that is to say, the lower the molecular weight, the higher the impregnation. However, when the differences in the molecular weight were low, as

between hydroxycinnamates and their corresponding hydroxycinnamics acids, its effect on D_e was not clearly distinguished.

By comparing D_e of total and individual phenolics, it may be inferred that low molecular weight phenolics (in the range of the ones investigated) are those mostly penetrating the model food in the operating conditions considered. In all cases, the D_e of total phenolics, a complex mixture of compounds with molecular weights ranging from one hundred to several thousand, was within the D_e range of individual phenolics and higher than the D_e of rutin.

With regard to solute transport in gels, only the values of sucrose D_e have been reported. Emam-Djomeh et al. (2001) obtained a sucrose D_e of 1.8 10^{-10} m^2/s for a 3% (w/w) agar gel during OD with a 32% sucrose solution by means of a diffusion cell. These data show a sucrose D_e that is higher than those obtained for most individual phenolics even though some of them have a molecular weight that is lower than that of sucrose (342 g/mol). During OD with 40 and 50º Brix red must, only caffeic acid had a D_e that was very similar to that reported for sucrose. However, even if molecular weights are similar, the results obtained for different solutes and different osmotic solutions are difficult to compare. In this study we used red must, a complex multi component aqueous solution made up of three major solutes (fructose, glucose, and sucrose), not just an aqueous binary sucrose solution.

The ratio of dehydration to impregnation (D_{ew}/D_e) for total and individual phenolics is depicted in Figure 3.4.

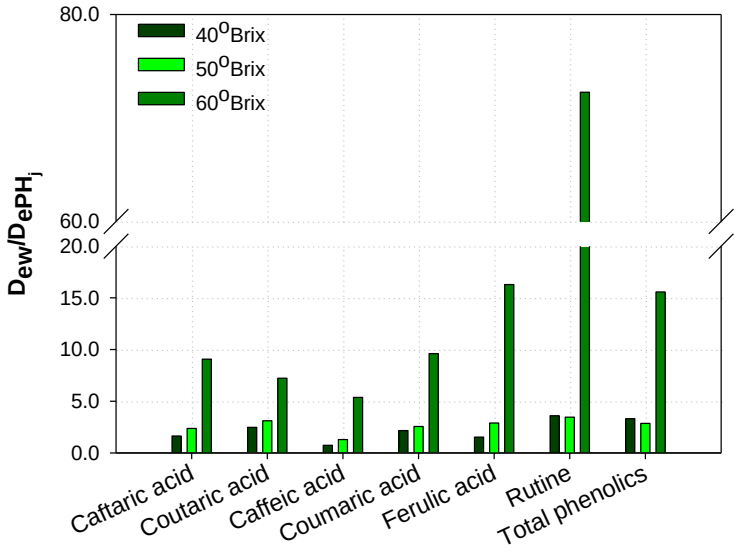

Figure 3.4. Dehydration to impregnation ratio (D_{ew}/D_e) against red must concentration.

This parameter clearly distinguished the main role of the soluble solids concentration of the red must in phenolic impregnation. Treatment with 60º Brix red must led to the largest difference between D_{ew} and D_e for individual and total phenolics with D_{ew}/D_e values between 1.2 and 72. In contrast, D_{ew}/D_e was not higher than 4 for any of the individual phenolics and total phenolics during OD with 40 and 50º Brix red must.

3.4. CONCLUSIONS

A significant mass transfer of total phenolics and low-molecular-weight phenolics (164 – 610 g/mol) was detected during OD of a model food (an agar-agar gel) with a concentrated red must of 40, 50 and 60º Brix. The progress of moisture, total phenolics, caftaric acid, coutaric acid, caffeic acid, coumaric acid, ferulic acid and rutin ratio showed that the concentration of soluble solids in the osmotic solution (red must) and the phenolics molecular weight controlled the rate of phenolic infusion.

Increasing the soluble solids concentration above a certain value (>50º Brix of red must) significantly decreased the penetration of total and individual phenolics in the model food. These results, in good agreement with those reported for several model (gel) and real foods, support the hypothesis that a highly concentrated layer of sugars develops on the food surface, hindering not only the impregnation of soluble solids but also the penetration of such other solutes as phenolics.

The molecular weight of the phenolics also had a significant impact on their infusion rate. The results showed that the penetration of phenolics of 612 g/mol was slightly lower in all conditions tested. In addition, by comparing the mass transfer of total to individual phenolics, we concluded that phenolics whose molecular weight was above 600 g/mol might make a poor contribution to total phenolic impregnation.

The effect of osmoactive solutes other than sucrose on phenolic impregnation during OD with phenolic-rich aqueous solutions should be studied so that the penetration rate of phenolics can be increased. In addition, of the solute-solute interactions during OD should be better characterized by determining not only phenolics but also the osmoactive solutes in the food impregnated. This may enable the operating conditions (composition of the osmotic solution, processing time) to be controlled better and products rich in antioxidants and appealing to food consumers to be formulated.

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CHAPTER 4

*COMMERCIAL EXTRACTS FROM GRAPE SEEDS AND MARC AS A SOURCE OF PHENOLICS TO ENRICH A MODEL FOOD**

* This chapter has been submitted to ***Journal of Food Engineering*** as: Rózek, A., Achaerandio, I., Güell, C., López, F., Ferrando, M. Use of commercial grape phenolic extracts to supplement solid foodstuff.

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4.1. INTRODUCTION

In recent years, interest has grown in developing new functional foods that have health-promoting and/or disease-preventing properties beyond the basic function of supplying nutrients. Since synthetic antioxidants raise toxicological concerns, grape extracts have become popular as nutritional supplements. Extracts from grapes contain a heterogeneous mixture of phenolic acids, simple flavonoids, complex flavonoids and anthocyanins. Commercial grape extracts are usually obtained from grape seeds and marc (i.e. solid residues of the wine-making process) in which the most abundant phenolics that have been isolated from grape seeds are catechin, epicatechin and procyanidins (Guendez et al., 2005, Shi et al., 2003, González-Paramás et al., 2004, Monagas et al., 2006, Rodríguez Montealegre et al., 2006). Numerous studies have demonstrated the benefits of the phenolic compounds in grapes, including antioxidant effects (Yilmaz et al., 2006, Amico et al., 2008), antimicrobial effects (Furiga et al., 2008; Sivarooban et al., 2008), anticancer effects (Athar et al., 2007, Morré et al., 2006) and protection against cardiovascular diseases (Pérez-Jiménez, 2008).

Osmotic treatment (OT) is widely used to modify the composition of solid foods (e.g. fruits, vegetables, meat and fish) by partially removing water and adding solutes. During immersion in a hypertonic (osmotic) solution, the higher osmotic pressure of the osmotic solution (OS) drives water transport from the solid food into the solution (Spiess & Behsnilian, 1998). Water transport is accompanied by the simultaneous counter-diffusion of solutes from the OS into the food structure. In the food industry, OT is used to improve quality in various areas, including colour, flavour, texture, energy efficiency and packaging (Rahman & Perera, 1999, Sablani et al., 2002).

Recently, Rózek et al. (2007) reported that OT is a unit operation suitable for introducing controlled quantities of solutes, such as grape phenolics with antioxidant properties. Using concentrated red grape must as a source of phenolics, they found that the phenolic content in an osmo-treated solid food (agar-agar gel) was in the same range as that of some phenolic-rich fruits. Nevertheless, in order to formulate a wide range of phenolic-supplemented solid food products with significant differences in composition, research is needed on other commercial sources of phenolics, such as extracts of grape seeds and skins.

The objective of this work was to determine how the phenolic profile and concentration of the osmotic solution (OS) and its osmotic pressure (i.e. water activity) adjusted by

sucrose content affects the end composition and antioxidant properties of a solid model food after OT. Two commercial extracts (from grape seed and white grape marc) with different phenolic profiles were used as sources of phenolics, and the sucrose content in the OS was set at either 50% or 0% in order to obtain high and low osmotic pressure gradients, respectively, between the model food and the OS. Two different models, a diffusional equation and Peleg's model, were used to simulate the mass transfer of the major phenolics infused into the model food during OT.

4.2. MATERIALS AND METHODS

4.2.1. Material

4.2.1.1. Osmotic solutions and model food

In the OS, two kinds of phenolic extracts were used as sources of phenolics: grape seed extract (GSE) and white grape marc extract (WGME) (Seppic, Paris, France). The osmo-active solute was sucrose (refined, 99.9%), and the osmotic pressure of the OS was adjusted by a 50% (w/w) sucrose solution. To determine the effect of the osmotic pressure gradient on phenolic infusion, some experiments were performed without sucrose in the OS. The total phenolic content of the various OSs used in the OT experiments was adjusted using GSE and WGME as shown in Table 4.1.

Table 4.1. Total phenolic content in the OSs with 50% and 0% sucrose

50% sucrose (w/w)		0% sucrose (w/w) (control)	
g GAE*/L	g GAE/kg	g GAE*/L	g GAE/kg
15.0 ± 0.4	12.4 ± 0.3	15.0 ± 0.4	15.0 ± 0.4
7.70 ± 0.5	6.17 ± 0.4	7.70 ± 0.5	7.70 ± 0.5
3.50 ± 0.2	2.80 ± 0.2	3.50 ± 0.2	3.50 ± 0.2

*GAE = gallic acid equivalent.

The model food, an agar-agar gel, was prepared with 4% (w/w) agar-agar (Scharlau, Barcelona, Spain), 9.6% (w/w) sucrose and distilled water. The mixture was heated to 95°C in a microwave oven until the agar-agar had dissolved completely. Gelation was achieved by cooling at room temperature. The gel was then stored at 6° ± 2°C prior to use within two days.

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4.2.2. Methods

4.2.2.1. Osmotic treatment

The experimental set-up, designed according to the specifications for OT equipment described by Marouzé et al. (2001), consisted of two parts: a perforated basket where the samples (around 50 g) were placed and a vessel filled with the OS (1 L). Agar-agar gel cubes (1 cm side) were weighed, placed in the basket and immersed in the corresponding OS. The cubes were then removed from the OS at different time intervals (0.5, 1, 2, 4 and 8 hours). After that, the samples were blotted in order to remove the excess OS from the surface and weighed using the analytical balance. The solution/food ratio was kept above 20 in order to guarantee that the concentration change in the osmotic media was always negligible. All experiments were run under atmospheric pressure and at room temperature.

4.2.2.2. Analytical determinations

4.2.2.2.1. Determination of moisture content

The moisture content of the fresh and osmo-treated food was determined gravimetrically according to AOAC method 934.06. Each determination was performed in duplicate.

4.2.2.2.2. Determination of soluble solids content

The osmo-treated model food was ground finely. Approximately 5 g of it was dissolved in 100 mL of Milli-Q water and placed on the magnetic stirrer for two hours. The mixture was then filtered and 1 mL was taken for sugar content determination. The total sucrose content was determined according to Rebelein's method (Barceló, 1990) using a sugar-analysis kit (GAB Sistemática Analítica, S.L., Barcelona, Spain).

4.2.2.2.3. Determination of total and individual phenolic content

In order to determine the total and individual phenolic content in the osmo-treated food, a sequential extraction was carried out according to the method reported by Rózek et al. (2007). The total phenolic content of the filtered and diluted extracts was then determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). The results were expressed as mg of gallic acid equivalents (GAE) per kg on a wet basis.

The individual phenolic content was determined using a high-performance liquid chromatography (HPLC) method. Before the analysis, the solvents were evaporated under low pressure at <40°C, re-suspended in a mixture of aqueous ethanol (15%, w/w) and filtered through 0.45 µm syringe filters (Teknokroma, Barcelona, Spain). The HPLC determinations were done using a Hewlett-Packard computer (HP/Agilent, Waldbronn, Germany), which was equipped with ChemStation software and coupled to a Supelcosil LC-18 column (25 cm x 4.6 mm) with a particle size of 5 µm. Peaks were monitored with a diode array detector (DAD), identified by their retention times and UV-Vis spectra, and quantified using a calibration curve of the corresponding standard compound. The DAD was used to choose the maximum absorbance for each group of compounds, to control peak purity and to identify the spectra of certain phenolics (Bétes-Saura et al, 1996).

The following standards were purchased from Sigma-Aldrich (Steinheim, Germany):

- Hydroxybenzoic acids: gallic acid, GA (170.12 g/mol).
- Flavan-3-ol monomers: (+)-catechin, CT (290.27 g/mol); (-)-epicatechin, ECT (290.27 g/mol); (-)-epicatechin 3-*O*-gallate, ECG (442.37 g/mol); (-)-epigallocatechin 3-*O*-gallate, EGCG (458.37 g/mol); and (-)-epigallocatechin, EGC (306.27 g/mol).
- Flavan-3-ol dimers: procyanidin B1, PAB1 (578.5 g/mol); and procyanidin B2, PAB2 (578.5 g/mol).

All determinations were performed in duplicate. The results were expressed as mg of phenol per kg on a wet basis.

4.2.2.2.4. Determination of antioxidant capacity: TEAC method

The antiradical scavenging capacity of gel extracts was assessed according to the ABTS decolorization assay (Re et al., 1999). This assay is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), which has a characteristic long wavelength absorption spectrum showing maxima at 734 nm. The results were expressed as the Trolox equivalent in mmol per kg on a wet basis. Each determination was performed in duplicate.

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4.2.3. Calculation procedures

4.2.3.1. Mass exchange calculations

Mass exchange between the solution and the gel sample during OT was evaluated using parameters such as water loss ($-\Delta M^{w}$) and sucrose gain (ΔM^{SUC}). These parameters were calculated according to the following equation:

$$\Delta M^{j} = \frac{M_t \cdot x_t^j - M_0 \cdot x_0^j}{M_0} \qquad (4.1)$$

where *M* and *x* are the mass of the gel and the mass of each component in the gel, respectively, the subscripts *0* and *t* indicate initial conditions and conditions at time *t* of treatment, respectively, and the superscript *j* indicates each of the aforementioned components. The mass fraction of each component in the gel is expressed as kg/kg on a wet basis. The amount of insoluble solids in the initial sample was assumed to be a fixed amount of the total initial solids and to remain constant throughout OT.

4.2.3.2. Peleg's model

The model proposed by Peleg (1988) and redefined by Palou et al. (1994) was used to fit the experimental results. Peleg's equation is an empirical model with two initial parameters that describes sorption isotherms approaching equilibrium asymptotically:

$$\frac{t}{x_t^j - x_0^j} = k_1^j + k_2^j t \qquad (4.2)$$

where *x* is the mass fraction of each component expressed as kg/kg on a wet basis, the subscripts *0* and *t* indicate initial conditions and conditions at time *t* of treatment, respectively, and the superscript *j* indicates each of the components transferred during OT. The constants k_1 and k_2 are the Peleg rate constant and Peleg capacity constant, respectively. The Peleg rate constant, k_1, relates to the initial mass change of any component:

$$\left.\frac{dx_t^j}{dt}\right|_{t=0} = \pm\frac{1}{k_1^j} \qquad (4.3)$$

The Peleg capacity constant, k_2, relates to the contents at equilibrium: the minimum attainable moisture content or maximum sucrose and phenolic contents. As time $t \to \infty$, Equation 4.2 gives the relation between equilibrium (∞) conditions and k_2.

$$x_\infty^j = x_0^j \pm \frac{1}{k_2^j} \qquad (4.4)$$

Peleg constants (k_1 and k_2) were obtained by regressing experimental data to equation (4.2).

4.2.3.3. Determination of effective diffusion coefficients

The samples were assumed to behave like isotropic structures and the mass transfer was assumed to be negligible as compared to the internal resistance. The solution of Fick's second law for cubic geometry was used to model the mass transfer of water and soluble solids during OT. The initial and boundary conditions assumed that the initial moisture and soluble solids content of the samples was uniform, that the solid food was symmetrical with regard to the mass transfer direction, and that the sample surface was in thermodynamic equilibrium. The mass transfer was assumed to occur between the food liquid phase (i.e. food containing water and soluble components) and the OS. Therefore, the equilibrium criterion was that the food liquid phase and the OS are compositionally equal (Barat, Fito & Chiralt, 1998). The effective diffusion coefficients were calculated by means of the analytical solution of Fick's second law applied to a cubical configuration. The total amount of each diffusing substance crossing the cube surface during a period of time *t* is given by Crank (1995).

$$Y_r^j = \frac{\left(z_t^j - z_\infty^j\right)}{\left(z^j - z_\infty^j\right)} = \left\{\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-(2n+1)^2\frac{\pi^2 D_{ej}}{4a^2}t\right]\right\}^3 \qquad (4.5)$$

where Y_r^j indicates the ratio of each diffusing compound or group of compounds; *z* is the mass fraction in the food liquid phase; the subscripts 0,∞, and *t* represent the initial

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concentration, the concentration at equilibrium, and the concentration at any time, respectively; D_e is the effective diffusion coefficient; and a is half of the thickness of the cube.

The values of D_e were determined by regressing experimental data to Equation 4. The fitting was performed with SigmaPlot version 10.0 by applying the Marquardt-Levenberg algorithm. This algorithm searches for the values of the parameter D_e that minimize the sum of the squared differences between the values of the observed and predicted values of the dependent variable. The regression coefficient, R^2, was used to measure how well the regression model described the data.

4.3. RESULTS AND DISCUSSION

4.3.1. Phenolic profile of grape seed and white grape marc extracts

Figure 4.1 shows the phenolic composition of GSE and WGME. Since low-molecular-weight phenolics have been found to infuse most easily into model food structure during OT (Rózek et al., 2007), phenolics with a molecular weight of less than 500 g/mol were identified and quantified.

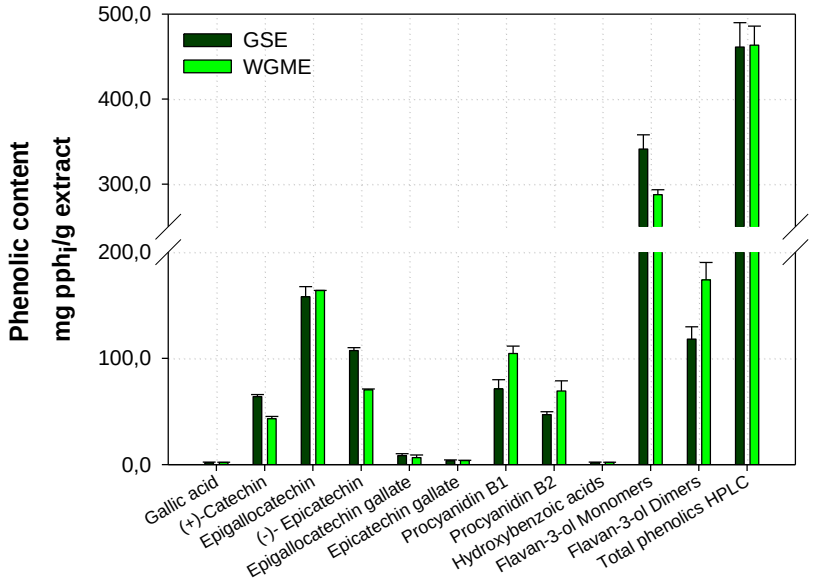

Figure 4.1. Content of individual phenolics determined by HPLC in GSE and WGME. Results expressed as mg of phenolic compound(s) per g of extract (d.w.).

The total phenolic content determined by HPLC for both extracts accounts for nearly 45% of the total phenolic content determined by the Folin-Ciocalteu's method. Of the

various polyphenols quantified, flavan-3-ol monomers were the largest group, followed by flavan-3-ol dimers and hydroxybenzoic acids. Overall, GSE was found to contain more flavan-3-ol monomers (74%) than does WGME (62%), whereas WGME was found to contain more flavan-3-ol dimers (37%) than does GSE (26%). Hydroxybenzoic acids were the smallest group of phenolics identified. Both extracts contained less than 1% of hydroxybenzoic acids (0.3% and 0.4% for GSE and WGME, respectively). In both extracts, the most abundant phenolics were EGC, ECT, CT and PAB1. Hydroxybenzoic acid (e.g. gallic acid) was present in the lowest concentration.

4.3.2. Phenolic profile of osmo-treated food and its antioxidant capacity

Figure 4.2 shows water loss ($-\Delta M^{w}$) and sucrose gain (ΔM^{suc}) during OT with sucrose and both grape extracts. After 8 h of OT with a 50% (w/w) sucrose solution in the OS, the largest water losses in the OS were 0.24 ± 0.02 for GSE and 0.26 ± 0.02 for WGME. Under the same conditions, the largest sucrose gains in the OS were 0.33 ± 0.01 for GSE and 0.32 ± 0.01 for WGME. A significant gain in phenolics was obtained during OT in all of the conditions considered.

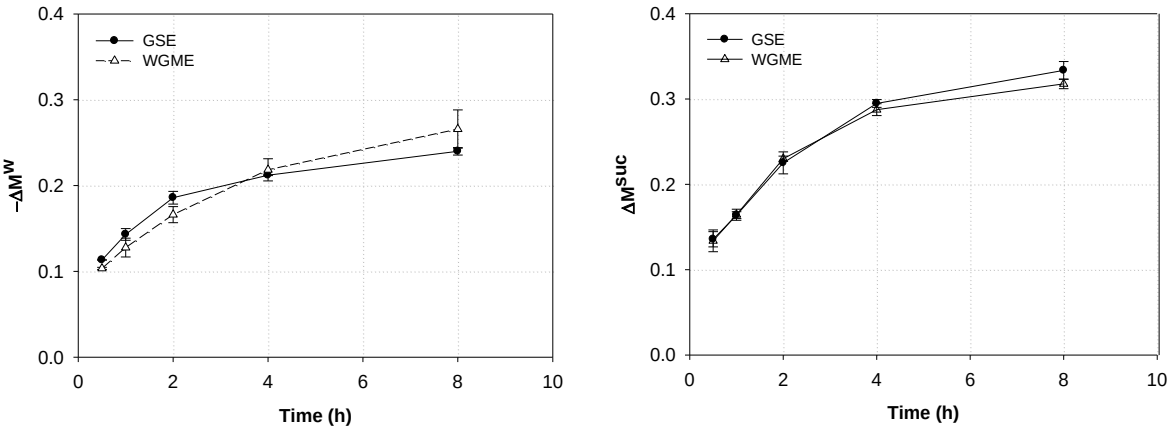

Figure 4.2. Mass loss and sucrose increment during OT with 50% sucrose.

With 50% (w/w) sucrose, the highest total phenolic content in the osmo-treated food was obtained after 8 h of OT and 15 g/L of total phenolics in the OS: 7176 ± 52 mg GAE/kg for GSE and 6458 ± 32 mg GAE/kg for WGME (Figure 4.3). When sucrose was not present in the OS, the total phenolic content was almost twice that obtained with 50% (w/w) sucrose in the OS (Figure 4.3).

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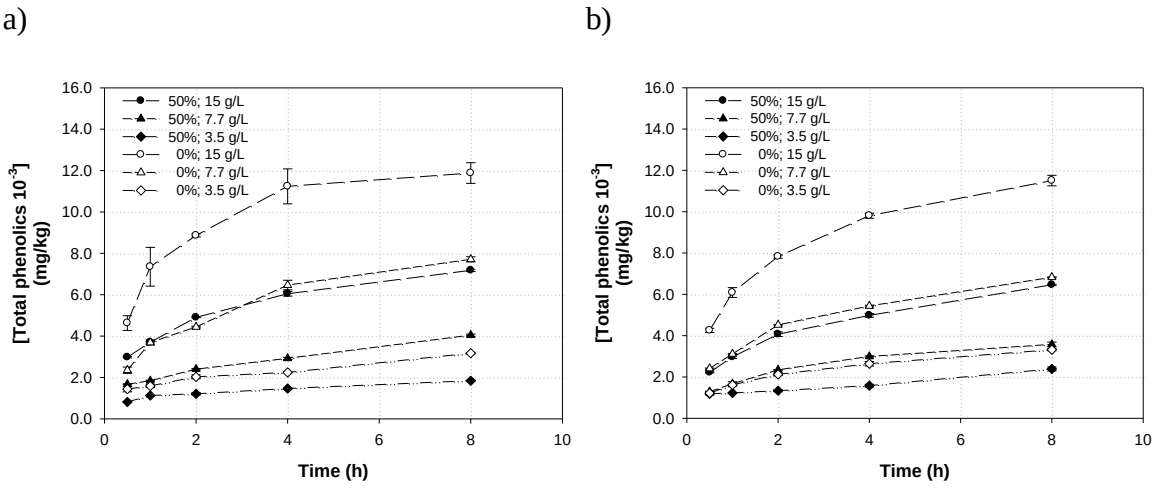

Figure 4.3. Total phenolic content in the model food during OT with a) GSE and b) WGME.

The phenolic concentration in the OS had a significant influence on the phenolic infusion into the model food. In order to formulate phenolic-rich solid foods, the operating time and the total phenolic and sucrose content in the OS must be optimised. In particular, in order to obtain an osmo-treated model food with a total phenolic content similar to that of the richest fruit - for instance, 5272 mg GAE/kg, which is a typical value for cranberries (Sun et al., 2002) - without changing its water content, several conditions can be set: 0.5 h of OT with 15 g GAE/L in the OS, or 3 h of OT with 7.7 g GAE/L and non-osmo-active solute in OS. In order to achieve not only phenolic infusion but also partial dehydration and sucrose impregnation, the following conditions can be set: 8 h of OT with 15 g GAE/L and 50% sucrose in the OS. These conditions led to a maximum total phenolic content of 4000 mg GAE/kg.

Of the two grape extracts used as sources of phenolics under the same conditions (i.e. total phenolic content and osmotic pressure of the OS), GSE yielded a higher phenolic gain in the osmo-treated model food. The differences between GSE and WGME in individual phenolic composition may explain these results (Figure 4.1). WGME is richer in flavan-3-ol dimers, whereas GSE contains more flavan-3-ol monomers. The latter are better at penetrating the model food during OT due to their lower molecular weight (Rózek et al., 2007). Depending on the source of phenolics used, different profiles of individual phenolics are obtained in the osmo-treated model food. For all of the conditions tested, the hydroxybenzoic acid, flavan-3-ol monomers and flavan-3-ol dimers quantified in the OS were found in the osmo-treated food (Figures 4.4, 4.5 & 4.6).

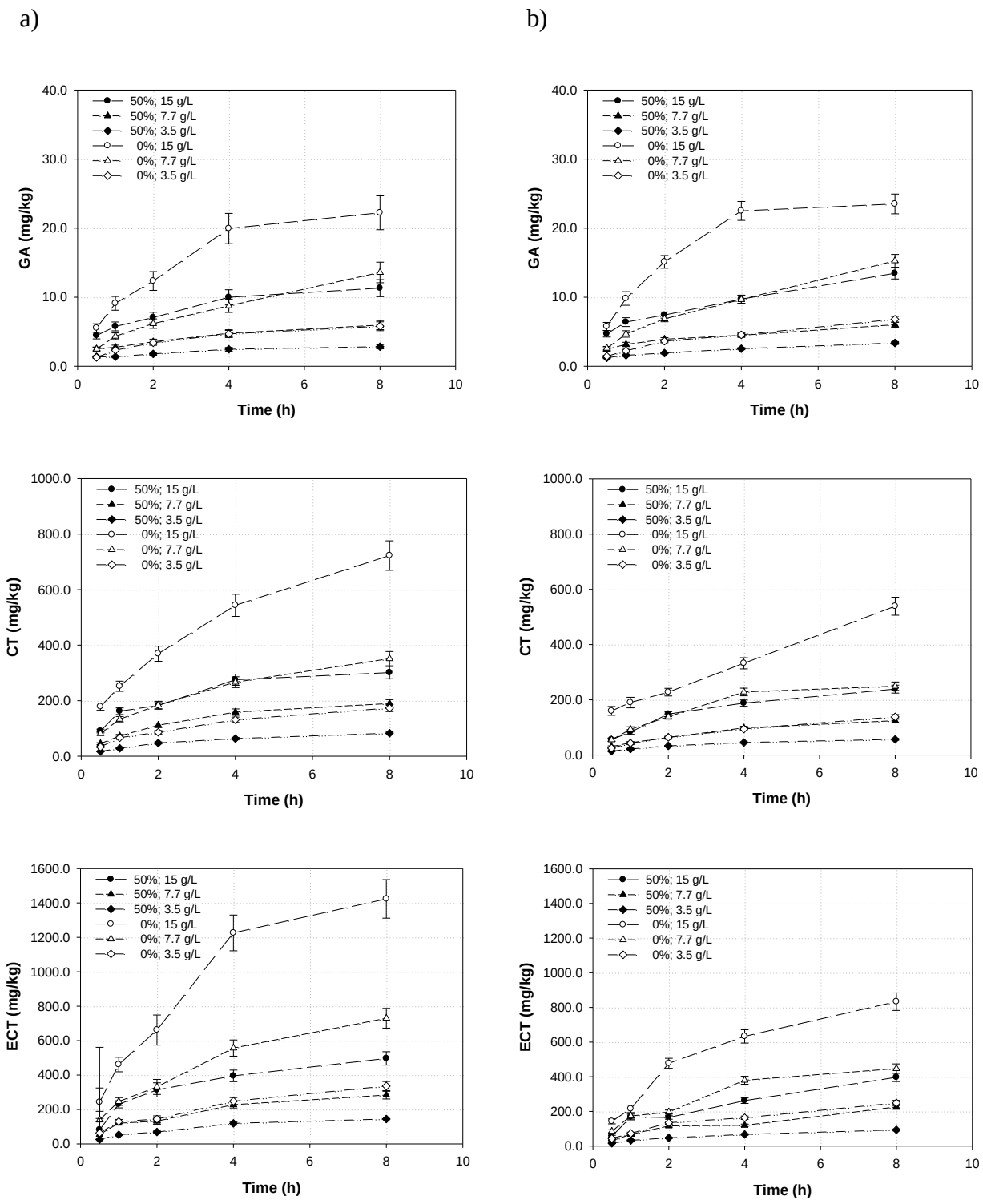

Figure 4.4. Content of gallic acid and the flavan-3-ol monomers (+)-catechin and (-)-epicatechin in the model food during OT with a) GSE and b) WGME.

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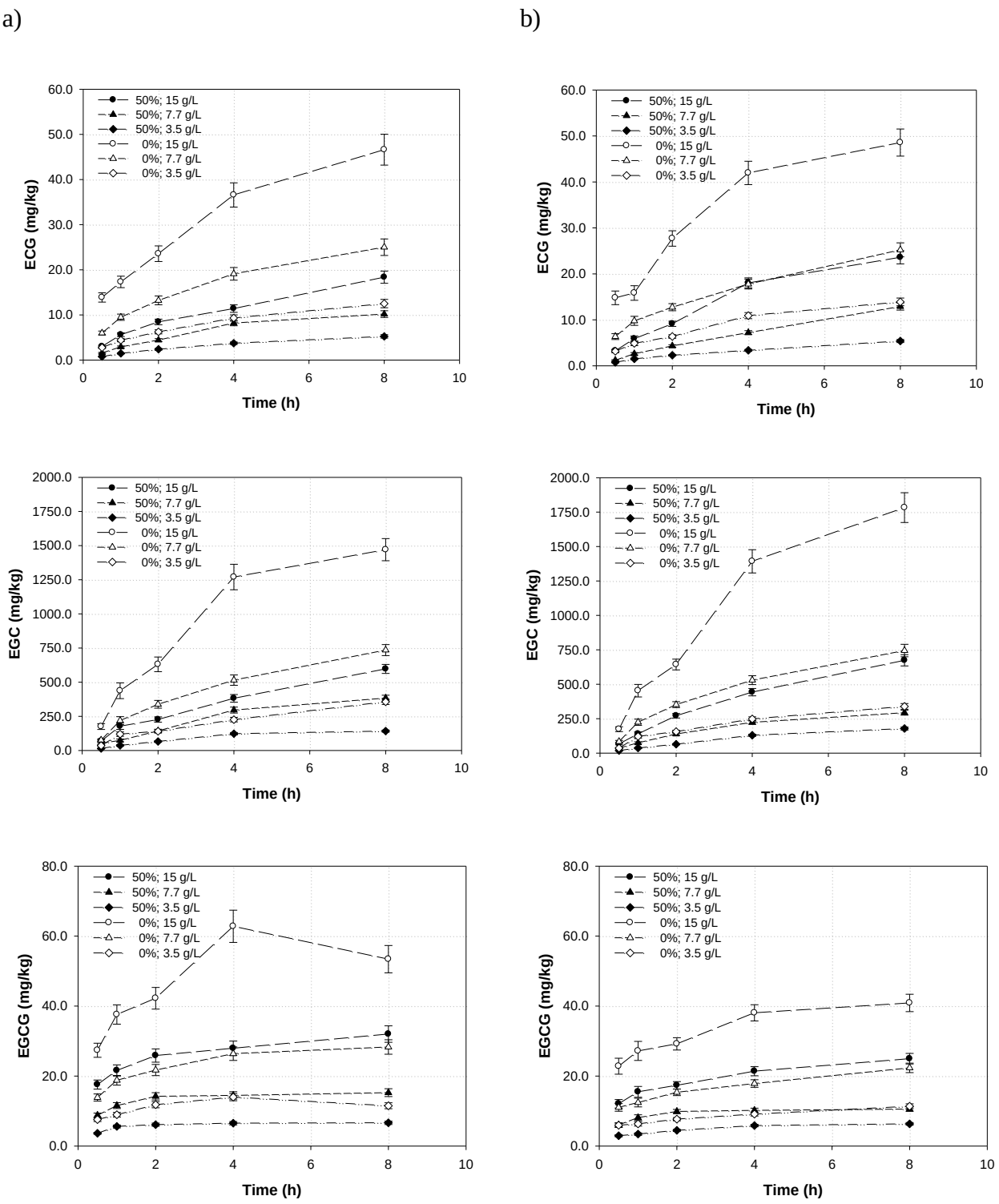

Figure 4.5. Content of the flavan-3-ol monomers ECG, EGC and EGCG in the model food during OT with a) GSE and b) WGME.

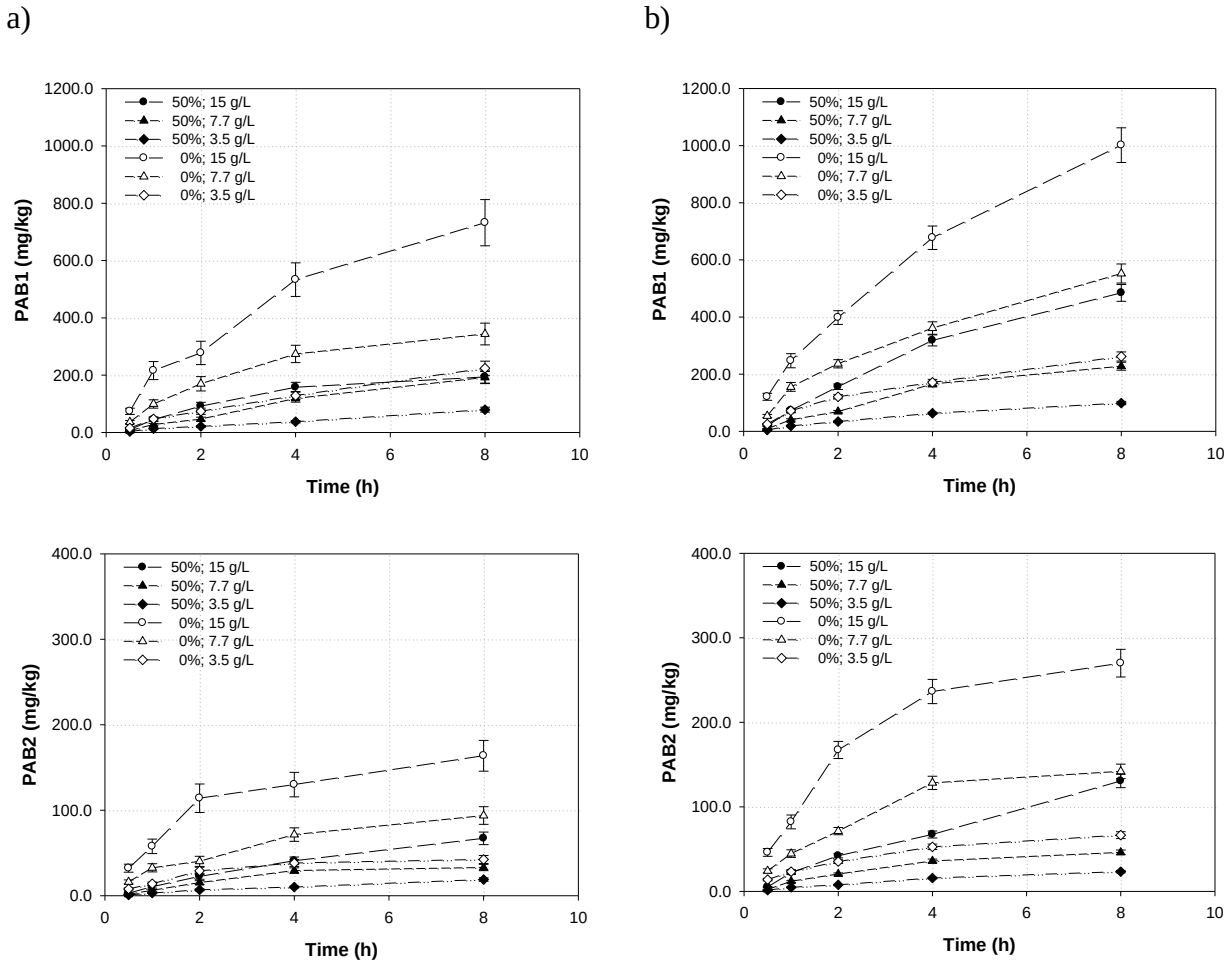

Figure 4.6. Content of flavan-3-ol dimers in the model food during OT with a) GSE and b) WGME.

Of all of the individual phenolics determined in the osmo-treated food, flavan-3-ol monomers - in particular EGC - were found in the highest concentration, since they were the most abundant phenolics found in GSE and WGME. However, CT, ECT and EGCG were found in higher concentrations in the model food that had been osmo-treated with GSE, while the flavan-3-ol dimers, PAB1 and PAB2, were found in higher concentrations in the model food that had been osmo-treated with WGME.

The antiradical scavenging capacity of the osmo-treated food was determined using the trolox-equivalent antioxidant capacity (TEAC) method. As observed with the total and individual phenolic content, the TEAC increased with processing time and with the total phenolic content in the OS (Figure 4.7). As a result, the TEAC was highest when a non-osmo-active solute OS and 15 g/L of total phenolics were used in the OS. Under these conditions, and after 8 h of OT, TEAC values were 127 ± 2.3 and 114 ± 2.4 mmol of Trolox/kg of FW for GSE and WGME, respectively. TEAC values of 20.24, 16.79 and 10.94 mmol of Trolox/kg of FW have been reported for blackberry, raspberry and

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strawberry, respectively (Pellegrini et al, 2003). Therefore, by using OT under the conditions that led to the highest phenolic gain, it was possible to obtain a product with a TEAC value six times higher than that observed in the fruits with the highest antioxidant capacity. Lower, but still significantly high, TEAC values were observed in food that had been osmo-treated with 50% sucrose in the OS: 74 and 61 mmol of Trolox /kg FW after 8 h of OT with GSE and WGME, respectively.

a) b)

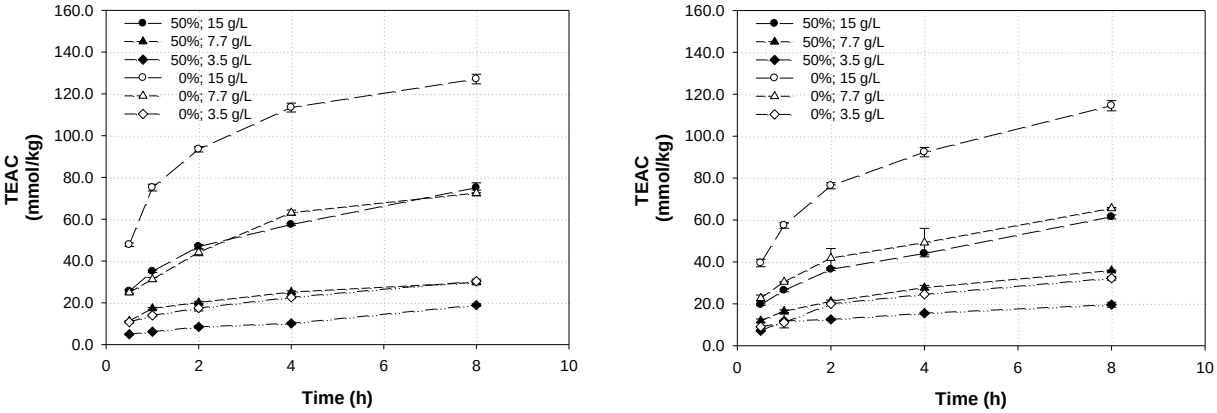

Figure 4.7. TEAC in the model food during OT with a) GSE and b) WGME.

The experimental values and corresponding linear regressions between TEAC and total phenolic content determined by HPLC for 50% solution and 0% sucrose are presented in the Figure 4.8.

a) b)

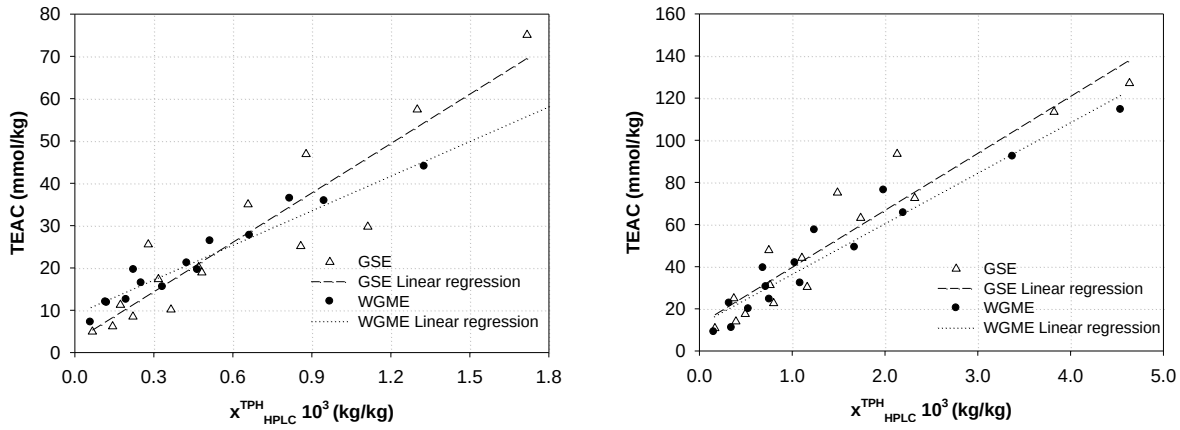

Figure 4.8. Correlation between TEAC and total phenolic content determined by HPLC for a) 50 % sucrose solution and b) Control solution.

The linear regression analysis (Table 4.2) showed that TEAC of the osmo-treated food is significantly correlated with the content of total phenolics determined by HPLC (i.e. calculated as the addition of each individual phenolic analysed). The slope values of linear regressions corresponding to OT with GSE were significantly higher than those obtained with WGME in OS either with 50% or 0% sucrose. These results show that, from both sources of grape phenolics used during OT, GSE contributed more effectively than WGME to TEAC increase since OT with GSE led to the highest ratio of TEAC to the amount of phenolic infused.

Table 4.2. Linear regression analysis of antioxidant capacity, TEAC, versus the total phenolic content in the osmo-treated food determined by HPLC

Composition of OS	a	b	R^2	P
50 % Sucrose (w/w)				
GSE	38934 ± 4350	2.6775 ± 3.3102	0.860	<0.0001
WGME	27280 ± 1336	9.1447 ± 1.0160	0.969	<0.0001
0 % Sucrose (w/w)				
GSE	27052 ± 2518	12.6173 ± 4.8743	0.899	<0.0001
WGME	23996 ± 1855	12.7720 ± 3.3672	0.928	<0.0001

a (mmol Trolox/kg of TPH) and
b (mmol of Trolox/kg) are the slope and the intercept, respectively.

4.3.3. Mass transfer of grape phenolics during OT: diffusional approach versus Peleg's model

Peleg's model (Equation 4.2) and the solution of Fick's equation for a cubical configuration (Equation 4.5) were used to describe the mass transfer of the solutes present in the multi-component OS during OT. In order to calculate effective diffusion coefficients, the changes in moisture, the soluble solids, and the total and individual phenolics in the model food were calculated as dimensionless values related to the initial and equilibrium conditions. The diffusion coefficients of water and sucrose were calculated only for the OT performed with 50% sucrose solutions. Because the osmotic pressure gradient between the OS and the model food was kept constant, similar rates of water diffusion were observed for GSE and WGME (moisture D_e 15.3 10^{-11} and 15.3 10^{-11} m^2/s, respectively). The sucrose diffusion rate was slightly lower than that of water (sucrose D_e 14.7 10^{-11} m^2/s) for both extracts. The diffusion coefficients of the total and individual phenolics determined by the Folin and HPLC methods, respectively, were lower than those of water and sucrose. With 50% (w/w) sucrose content in the OS, the D_e of the total phenolics (6.76 10^{-11} for GSE and 7.39 10^{-11} m^2/s for WGME) was

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greater than the D_e of the individual phenolics. Furthermore, the D_e of GA ($4.41\ 10^{-11}$ for GSE and $4.08\ 10^{-11}$ m^2/s for WGME) was higher than that of the flavan-3-ol monomers and the flavan-3-ol dimers (Table 4.3). During OT with the control solution (0% osmo-active solute), no dehydration effect was observed. Therefore, only the effective diffusion coefficients of the transferred solutes were calculated. Under these conditions, the D_e values of the total phenolics and of each individual phenolic (Table 4.4) were significantly higher than in the OT with sucrose solution.

Table 4.3. Effective diffusion coefficients of moisture, sucrose and total and individual phenolics during OT with 50% sucrose and GSE or WGME as a source of phenolics

	GSE		WGME	
	$D_e\ 10^{11}$ m^2/s	R^2	$D_e\ 10^{11}$ m^2/s	R^2
moisture	15.3 ± 0.17	0.946	15.3 ± 2.39	0.949
sucrose	14.7 ± 0.45	0.941	14.7 ± 2.36	0.944
total phenolics $_{FC}$	6.76 ± 1.30	0.904	7.39 ± 0.76	0.928
GA	4.41 ± 0.60	0.881	4.08 ± 0.74	0.909
CT	2.33 ± 0.56	0.839	2.45 ± 0.66	0.838
EGC	n.s. *	n.s.	n.s.	n.s.
ECT	1.98 ± 0.50	0.798	2.17 ± 0.67	0.804
EGCG	n.s.	n.s.	1.21 ± 0.40	0.725
ECG	2.16 ± 0.65	0.801	2.37 ± 0.83	0.776
PAB1	n.s.	n.s.	n.s.	n.s.
PAB2	n.s.	n.s.	n.s.	n.s.

* n.s. = not significant at a confidence level of $P<0.05$.

Table 4.4. Effective diffusion coefficients of moisture, sucrose and total and individual phenolics during OT with 0% sucrose and GSE or WGME as a source of phenolics

	GSE		WGME	
	$D_e\ 10^{11}$ m^2/s	R^2	$D_e\ 10^{11}$ m^2/s	R^2
total phenolics $_{FC}$	23.1 ± 2.82	0.935	20.1 ± 3.26	0.955
GA	13.9 ± 3.72	0.922	13.6 ± 3.01	0.914
CT	8.36 ± 0.75	0.919	10.6 ± 2.38	0.902
EGC	4.45 ± 0.49	0.825	4.84 ± 1.20	0.836
ECT	12.5 ± 0.65	0.891	11.1 ± 2.70	0.883
EGCG	3.83 ± 0.34	0.750	3.47 ± 0.77	0.807
ECG	14.9 ± 0.72	0.926	14.9 ± 2.73	0.923
PAB1	6.03 ± 1.50	0.841	5.11 ± 1.43	0.843
PAB2	n.s. *	n.s.	n.s.	n.s.

* n.s. = not significant at a confidence level of $P<0.05$.

Fick's model was used to describe the mass transfer of grape phenolics. The values of the regression coefficient (R^2) in the OT simulation with 0% sucrose were higher than those obtained during OT with 50% sucrose in the OS. Furthermore, the changes in the EGC, EGCG, PAB1 and PAB2 content during OT with 50% sucrose in the OS did not fit the diffusional model. These differences are due to the different mechanisms underpinning mass transfer with and without sucrose. OT with a non-osmo-active solute

in the OS gives rise only to a flow of solutes (grape phenolics) from the solution into the food (as opposed to counter-current flows of water and solutes, typical during OT), which is better described by the diffusional model. This equation (4.5) does not consider changes in the volume of the food due to water removal, which occur during OT with non-osmo-active solute in the OS but not during OT with 50% sucrose in the OS.

Peleg's kinetic model was also used to describe mass transfer over processing time. Tables 4.5 and 4.6 show the values of the Peleg constants (k_1 and k_2) for various sucrose and phenolic concentrations in the OS. Experimental data on phenolic content during OT fit well to Peleg's model under nearly all of the experimental conditions considered (R^2>0.97). The only changes that did not fit Peleg's equation were PAB1 and PAB2 content during OT with 50% sucrose in the OS. Whereas the increase in EGCG during OT did not fit well to Fick's equation, it did fit well to Peleg's model both with and without sucrose (R^2>0.99).

Figure 4.9 shows the initial rate of phenolic mass transfer ($1/k_1$) and the equilibrium phenolic content ($1/k_2$) under each set of conditions. With 50% sucrose in the OS, the initial rate of phenolic mass transfer ($1/k_1$) was lower than that observed with 0% sucrose with either of the extracts. Similarly, the phenolic content at equilibrium ($1/k_2$) was significantly higher after OT with 0% sucrose. In addition, the concentration of total phenolics in the OS controlled the initial rate of phenolic mass transfer, since the $1/k_1$ values increased with the total phenolic content in the OS. The two sources of grape phenolics yielded different results. The values of $1/k_1$ were higher with GSE than with WGME, except in the case of flavan-3-ol dimers. According to the $1/k_2$ values, ECT, EGC and CT are the individual phenolics found at the highest concentrations in model food that has reached equilibrium after OT with GSE, whereas EGC, PAB1 and ECT have the highest concentrations in model food that has reached equilibrium conditions after OT with WGME.

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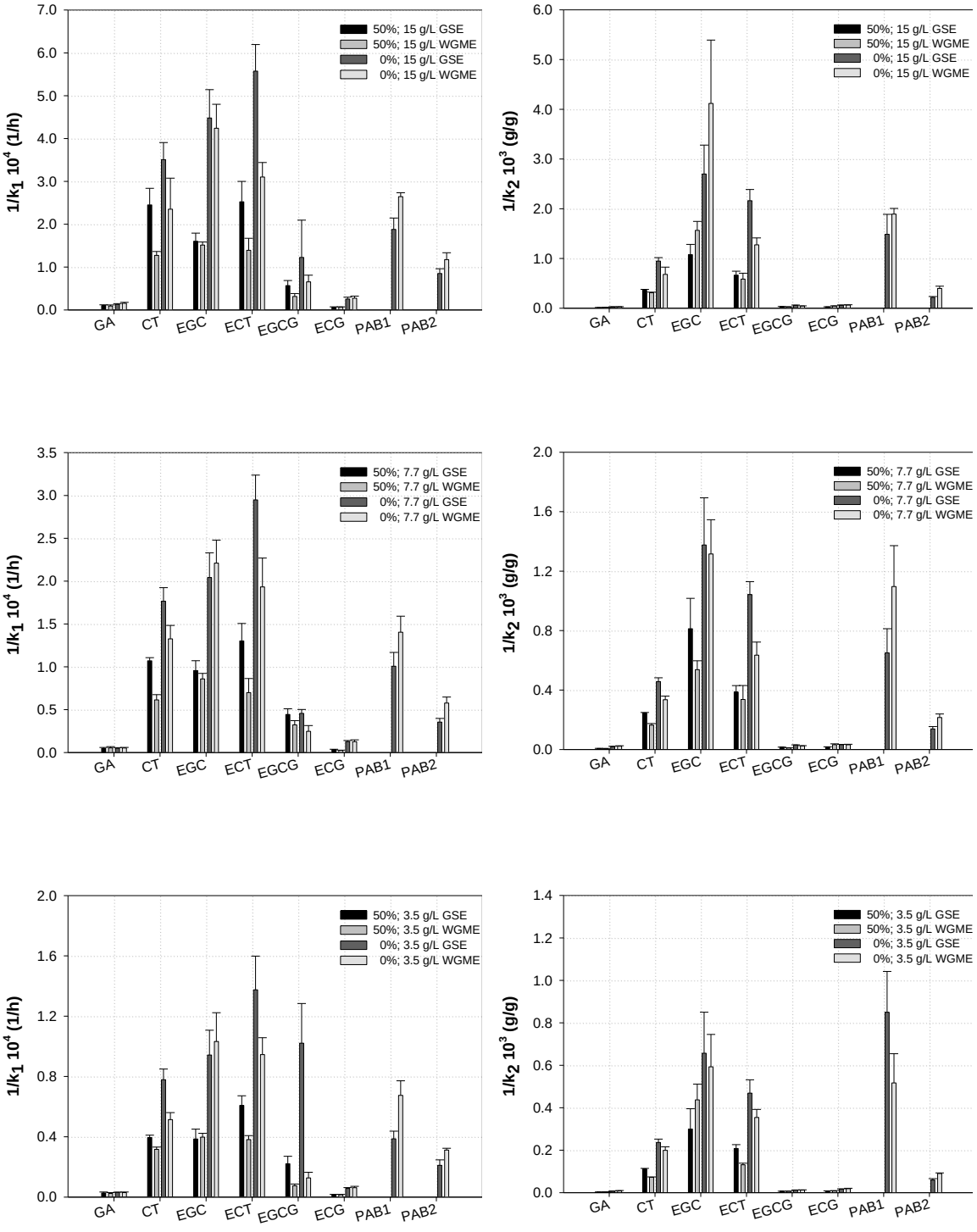

Figure 4.9. Initial rate of phenolic mass transfer ($1/k_1$) and equilibrium phenolic content ($1/k_2$) during OT with 50% and 0% sucrose and 15 g/L, 7.7 g/L, and 3 g/L of total phenolics using GSE or WGME as a source.

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Table 4.5. Peleg constants for OT with 50% sucrose

	Grape seed extract								
	15 g/L			7.7 g/L			3.5 g/L		
	k_1	k_2	R^2	k_1	k_2	R^2	k_1	k_2	R^2
Moisture	-3.59 ± 0.65	-3.06 ± 0.16	0.992	-3.78 ± 0.45	-3.07 ± 0.11	0.996	-4.18 ± 0.58	-2.94 ± 0.14	0.993
Sucrose	3.06 ± 0.52	2.88 ± 0.13	0.994	2.60 ± 0.29	3.09 ± 0.07	0.999	2.32 ± 0.39	3.11 ± 0.09	0.997
Total phenolics $_{FC}$	140.8 ± 19.8	123.8 ± 4.8	0.996	330.5 ± 96.9	217.4 ± 23.5	0.966	505.5 ± 139.7	497.2 ± 33.8	0.986
Individual phenolics	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2
GA	97.5 ± 15.9	76.8 ± 3.8	0.993	209.6 ± 46.7	146.0 ± 11.3	0.982	367.6 ± 82.5	312.1 ± 20.0	0.988
CT	4.1 ± 0.6	2.8 ± 0.2	0.991	9.3 ± 0.3	4.1 ± 0.1	0.999	25.3 ± 1.0	9.0 ± 0.2	0.998
EGC	6.2 ± 0.7	0.9 ± 0.2	0.900	10.5 ± 1.3	1.2 ± 0.3	0.841	26.0 ± 4.4	3.3 ± 1.1	0.765
ECT	4.0 ± 0.7	1.5 ± 0.2	0.958	7.7 ± 1.2	2.6 ± 0.3	0.964	16.4 ± 1.7	4.8 ± 0.4	0.978
EGCG	17.6 ± 3.6	29.5 ± 0.9	0.997	22.5 ± 3.3	62.7 ± 0.8	1.000	45.2 ± 10.2	144.1 ± 2.5	0.999
ECG	157.9 ± 20.5	37.2 ± 5.0	0.949	288.7 ± 24.3	60.6 ± 5.9	0.973	583.3 ± 15.1	118.7 ± 3.7	0.997
PAB1	n.s. *	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PAB2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

	White grape marc extract								
	15 g/L			7.7 g/L			3.5 g/L		
	k_1	k_2	R^2	k_1	k_2	R^2	k_1	k_2	R^2
Moisture	-5.3 ± 1.1	-2.8 ± 0.3	0.972	-4.8 ± 0.7	-2.9 ± 0.2	0.990	-4.4 ± 0.7	-2.9 ± 0.2	0.989
Sucrose	2.4 ± 0.3	3.0 ± 0.1	0.998	2.7 ± 0.3	3.1 ± 0.1	0.998	2.7 ± 0.4	3.1 ± 0.1	0.997
Total phenolics $_{FC}$	211.9 ± 35.0	132.1 ± 8.5	0.988	314.1 ± 28.0	250.6 ± 6.8	0.998	598.5 ± 156.6	363.0 ± 37.9	0.968
Individual phenolics	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2
GA	109.4 ± 28.8	64.0 ± 7.0	0.966	187.4 ± 53.1	149.7 ± 12.9	0.978	415.7 ± 95.9	256.0 ± 23.2	0.976
CT	7.9 ± 0.6	3.2 ± 0.1	0.995	16.3 ± 1.6	6.1 ± 0.4	0.988	31.6 ± 1.5	13.8 ± 0.4	0.998
EGC	6.6 ± 0.3	0.6 ± 0.1	0.962	11.6 ± 0.9	1.9 ± 0.2	0.964	25.2 ± 1.6	2.3 ± 0.4	0.920
ECT	7.2 ± 1.4	1.7 ± 0.4	0.888	14.3 ± 3.4	3.0 ± 0.8	0.812	26.2 ± 1.8	7.6 ± 0.4	0.990
EGCG	31.6 ± 6.6	36.8 ± 1.6	0.994	31.0 ± 4.7	90.6 ± 1.1	1.000	132.8 ± 19.9	141.4 ± 4.8	0.997
ECG	148.7 ± 12.5	23.2 ± 3.0	0.951	391.0 ± 23.5	30.9 ± 5.7	0.908	614.0 ± 58.0	115.6 ± 14.0	0.958
PAB1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PAB2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

* n.s. = not significant at a confidence level of $P<0.05$.

Peleg constants k_1 and k_2 are expressed in *h* and *kg/kg*, respectively.

Table 4.6. Peleg constants for OT with 0% sucrose

	Grape seed extract								
	15 g/L			7.7 g/L			3.5 g/L		
	k_1	k_2	R^2	k_1	k_2	R^2	k_1	k_2	R^2
Total phenolics $_{FC}$	51.8 ± 21.6	78.6 ± 5.22	0.987	183.8 ± 23.8	108.1 ±5.8	0.992	366.2 ±135.4	286.3 ± 32.8	0.962
Individual phenolics	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2
GA	76.1 ± 8.9	35.0 ± 2.2	0.989	199.2 ± 26.4	51.9 ± 6.4	0.957	316.1 ± 8.4	133.4 ± 2.0	0.999
CT	2.8 ± 0.3	1.1 ± 0.1	0.984	5.7 ± 0.5	2.2 ± 0.1	0.991	12.8 ± 1.2	4.2 ± 0.3	0.987
EGC	2.2 ± 0.3	0.4 ± 0.1	0.878	4.9 ± 0.7	0.7 ± 0.2	0.863	10.6 ± 1.9	1.5 ± 0.4	0.794
ECT	1.8 ± 0.2	0.5 ± 0.0	0.968	3.4 ± 0.3	1.0 ± 0.1	0.979	7.3 ± 1.2	2.1 ± 0.3	0.949
EGCG	8.2 ± 5.8	17.1 ± 1.4	0.980	21.9 ± 2.2	32.6 ± 0.5	0.999	9.8 ± 25.2	82.5 ± 6.1	0.984
ECG	39.3 ± 6.7	17.0 ± 1.6	0.974	77.9 ± 7.1	30.9 ± 1.7	0.991	173.9 ± 16.1	59.7 ± 3.9	0.987
PAB1	5.3 ± 0.8	0.7 ± 0.2	0.820	9.9 ± 1.6	1.5 ± 0.4	0.842	n.s. *	n.s.	n.s.
PAB2	11.8 ± 1.5	4.6 ± 0.4	0.981	28.2 ± 3.4	7.2 ± 0.8	0.963	47.4 ± 8.0	16.9 ± 1.9	0.962

	White grape marc extract								
	15 g/L			7.7 g/L			3.5 g/L		
	k_1	k_2	R^2	k_1	k_2	R^2	k_1	k_2	R^2
Total phenolics $_{FC}$	120.9 ± 29.4	57.6 ± 7.1	0.956	183.6 ± 26.0	126.5 ± 6.3	0.993	364.4 ± 58.7	262.9 ±14.2	0.991
Individual phenolics	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2	$k_1 10^{-3}$	$k_2 10^{-3}$	R^2
GA	64.2 ± 9.0	64.2 ± 9.0	0.987	188.1 ± 23.5	188.1 ± 23.5	0.954	339.2 ± 47.0	339.2 ± 47.0	0.969
CT	4.3 ± 1.3	4.3 ± 1.3	0.878	7.5 ± 0.9	7.5 ± 0.9	0.985	19.5 ± 1.7	19.5 ± 1.7	0.979
EGC	2.4 ± 0.3	2.4 ± 0.3	0.777	4.5 ± 0.5	4.5 ± 0.5	0.916	9.7 ± 1.8	9.7 ± 1.8	0.834
ECT	3.2 ± 0.4	3.2 ± 0.4	0.966	5.2 ± 0.9	5.2 ± 0.9	0.945	10.6 ± 1.3	10.6 ± 1.3	0.967
EGCG	15.3 ± 3.6	15.3 ± 3.6	0.996	40.4 ± 11.0	40.4 ± 11.0	0.988	78.5 ± 22.7	78.5 ± 22.7	0.986
ECG	36.4 ± 6.4	36.4 ± 6.4	0.973	78.9 ± 13.2	78.9 ± 13.2	0.970	159.7 ± 22.5	159.7 ± 22.5	0.969
PAB1	3.8 ± 0.1	3.8 ± 0.1	0.989	7.1 ± 0.9	7.1 ± 0.9	0.841	14.8 ± 2.1	14.8 ± 2.1	0.825
PAB2	8.5 ± 1.2	8.5 ± 1.2	0.964	17.3 ± 2.1	17.3 ± 2.1	0.965	32.1 ± 1.2	32.1 ± 1.2	0.998

* n.s. = not significant at a confidence level of P<0.05.

Peleg constants k_1 and k_2 are expressed in *h* and *kg/kg*, respectively.

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4.4. CONCLUSIONS

Intermediate-moisture phenolic-rich products can be formulated using an OS with a suitable composition (i.e. grape phenolic concentration and sucrose content). Although the source of the grape phenolics and the phenolic content in the OS do influence the extent of phenolic infusion in a model food, the sucrose content in the OS is the factor that has the greatest impact. With a 50% sucrose concentration, a total phenolic content in the OS ranging from 3.5 g/L to 15 g/L, and an operating time of up to 8 h, the final total phenolic content in the osmo-treated model food was between 815 mg GAE/kg and 7176 mg GAE/kg. This is between 0.5 and 1.5 times higher than that of the richest fruits. OT with the same phenolic content but without sucrose in the OS led to a total phenolic content in the model food that was 80% to 100% higher.

The source of phenolics determines the total phenolic content, the individual phenolic profile and the TEAC of the osmo-treated model food. GSE, which has a higher concentration of flavan-3-ol monomers and a lower concentration of flavan-3-ol dimers than WGME, led to a higher content in total phenolics than that obtained with WGME. Consequently, the TEAC of the food treated with GSE was significantly higher than that of the food treated with WGME under the same conditions. Furthermore, the ratio of TEAC to the amount of infused phenolics was higher with GSE.

The changes in the total and individual phenolic content during OT without sucrose in the OS fit better to the diffusional model, whereas the kinetics of individual phenolic infusion during OT with 50% sucrose in the OS fit better to Peleg's equation. The changes in the flaval-3-ol dimers (PAB1 and PAB2) were the only changes that did not fit either model.

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CHAPTER 5

*MASS TRANSFER OF GRAPE PHENOLICS DURING OSMOTIC TREATMENT WITH DIFFERENT TYPES OF OSMO-ACTIVE SOLUTES**

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5.1. INTRODUCTION

By-products of the wine- and grape juice making industries (grape pulp, seeds, skins) are a good, inexpensive source of high-quality polyphenolic compounds, which is why the recovery of such compounds has been widely investigated (Balasundram et al., 2006; Lafka et al., 2007, Makris et al., 2007). Grape seeds and skins contain flavonoids (catechin, epicatechin, procyanidins and anthocyanins), phenolic acids (gallic acid, ellagic acid), stilbenes (resveratrol and piceid) and other phenolics. These constituents have been shown to have a beneficial effect on human health. In particular, phenolic compounds have a wide range of physiological properties: they are antibacterial (Özkan et al., 2004; Rodríguez Vaquero et al., 2005), antioxidant (Guendez et al., 2005; Spigno et al., 2007), anticancer (Morré, 2006), anti-inflammatory (Middleton et al., 2000), and antithrombotic (Folts, 1998), and they also play an important role in protecting against a number of pathological disturbances, such as artherosclerosis and brain dysfunction. Extracts from *Vitis vinifera* are commonly used to formulate dietary antioxidant supplements together with synthetic vitamins (E and C), minerals (selenium), soy isoflavones, tomato concentrate, rosemary extract, citrus flavonoids, and others (Monagas et al., 2006).

The development and consumption of functional foods, or foods that promote health not merely basic nutrition, is on the rise. Osmotic treatment (OT) seems to be a feasible technology for developing fruit and vegetable matrices to which functional ingredients can be successfully added to provide novel functional product categories and new commercial opportunities (Alzamora et al., 2005).

OT is a non-thermal treatment, the aim of which is to modify the composition of food material by partially removing water and impregnating it with solutes, without affecting the material's structural integrity (Spiess & Behsnilian, 1998). When samples are immersed in a hypertonic media, the osmotic solute is transported from the solution to the food material and water flows out of the product counter-currently. In OT, quality can be improved not only by removing water with the minimal thermal stress but also by impregnating the solutes and modifying the structure (Torreggiani & Bertolo, 2001). If the solutes are correctly chosen, and the ratio of water removal to solute impregnation is controlled, the natural flavor of fruit products and color retention can be enhanced. The two solute types most commonly used for osmotic treatments are sugars (mainly with fruits) and salts (with vegetables, fish, meat and cheese) with relevance for sucrose and

sodium chloride. The nature of the solute used, and hence its molecular or ionic behavior, strongly affects the kinetics of water removal and solute uptake. In recent years, multi-component solutions have been increasingly used to treat fruits (Sacchetti et al., 2001; Sereno et al., 2001) and meat and fish (Collignan & Raoult-Wack, 1992). Another osmo-active solute used in OT is glycerol, a food additive that improves the texture of foods (plasticizer) (Clubbs et al., 2005) and which can act as a microbiological protectant (Qiu & Bending, 2002). Recently, Rózek et al. (2007a) described OT as a suitable method for infusing solid foodstuffs with grape phenolics. They used an agar-agar gel as a model food and a concentrated red grape juice as the source of the phenolics. Under the conditions that maximized phenolic infusion, the total phenolic content of the gel was close to the values reported in some rich-in-phenolic fruits and vegetables, increasing, simultaneously, their antioxidant capacity.

The main objective of this study was to investigate how the nature of the osmo-active solute affects the infusion pattern of grape phenolics in a solid model food and the antioxidant properties of the model. To this end, mass transfer during OT of water, osmo-active solutes and total and some individual grape phenolics of low molecular weight, were characterized by two approaches: a diffusional model and the empirical Peleg's equation. A commercial extract of grape seed was the source of phenolics and an agar gel was the model solid food. The mass fraction of grape phenolics in the osmotic solution (OS) was kept constant, and sucrose, NaCl and glycerol were used as single osmo-active solutes or mixtures (NaCl and sucrose). The water activity of the osmotic solution was kept constant so that the driving force was the same in all cases. Effective diffusion coefficients and Peleg's constants of these major and minor components were used to evaluate how the type of osmo-active solute affected mass transfer in order to formulate solid food products supplemented with grape phenolics, when a complex multi-component mixture is used as osmotic solution.

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5.2. MATERIALS AND METHODS

5.2.1. Model food and osmotic solution

A model food of agar-agar gel was prepared with 4% (w/w) agar-agar (Scharlau, Spain), 9.6% (w/w) sucrose and distilled water. The mixture was heated to 95 °C in a microwave oven until the agar-agar was completely dissolved. Gelation was achieved by cooling to room temperature. The gel was then stored at 6 ± 2 °C and used within 2 days.

A multi-component aqueous solution consisting of one or two osmo-active solutes and a commercial grape seed extract (Vitisol® supplied by Berkem, Gardonne, France) was used as an osmotic solution. The osmo-active solutes used were: sucrose (refined, 99.9% sucrose), sodium chloride (J.T. Baker, Germany) and glycerol (Sigma-Aldrich, Steinheim, Germany). The osmotic pressure was adjusted so that water activity was constant (0.935 ± 0.010). The concentrations of sucrose, sodium chloride, and glycerol were 2.92m, 1.9m, and 4.65m, respectively. The mixture of sucrose/sodium chloride had a concentration of 1.46m sucrose and 0.95m sodium chloride. As a control, a solution with no osmo-active solute was used. The water activity (a_w) of solutions was measured at 25°C in an electric hygrometer (Novasina, IC-500, AW-LAB). In all experiments performed, the mass fraction of total phenolics was set at 6300 ± 45 mg GAE/kg.

5.2.2. Osmotic treatment

The experimental set-up consisted of two parts: a perforated basket in which gel samples were placed and a vessel that was filled with the osmotic solution. The basket had three shelves and guaranteed that the sample could be totally immersed in the osmotic solution. The osmotic solution was agitated by a magnetic stirrer. The agitation level was chosen so that the surface mass transfer was negligible. About 50 g of agar-agar gel cubes (1 cm per side) was weighed and placed in the OT basket. The basket was then submerged in 1 L of osmotic solution. The model food was processed for 0.5, 1, 2, 4 and 8 h. In all the experiments, the solution/gel ratio (w/w) was over 20 to avoid significant modifications to the concentration of the osmotic medium. During the experiment, the temperature was kept at 25 ± 2 °C and the setup was covered to minimize the effect of light. After the OT, the gel cubes were removed from the solution, drained, rinsed with distilled water, placed on absorbent paper to remove the excess of osmotic solution, and

weighed. Each experiment was carried out in duplicate. All experiments were run under atmospheric pressure.

5.2.3. Analytical methods

5.2.3.1. Determination of moisture and soluble solid content

The moisture content of fresh and osmo-treated food was determined gravimetrically with the 934.06 AOAC method. Each determination was made at least in triplicate.

Previous to determine sucrose and sodium chloride content, samples of about 5 g of milled fresh and osmo-treated gel were dissolved in 100 mL of Milli-Q water, placed on a magnetic stirrer, agitated for 2 hours and filtrated. The sodium chloride content was quantified according to Mohr's method, which consists of titration with 0.1M $AgNO_3$ (James, 1995). The total sucrose content was determined by the Rebelein method (Barceló, 1990) using a GAB kit for sugar analysis (GAB Sistemática Analítica S.L., Barcelona, Spain). The glycerol content of the osmo-dehydrated model food was calculated from the overall soluble solid gain after OT.

5.2.3.2. Extraction of phenolic compounds and soluble solids from the osmo-treated food

To determine the extent of phenolic impregnation in the model food after OT, sequential extraction was carried out (Rózek et al 2007). Then, filtrated and appropriately diluted extracts were taken for Folin-Ciocalteu's analysis, and TEAC and HPLC analysis. Prior to HPLC analysis the mixture was dried by vacuum distillation ($T \leq 40°C$), re-suspended in Milli-Q water and filtered through 0.45 µm syringe filters (Teknokroma, Barcelona, Spain). Samples were protected from light and high temperatures and were stored for short periods (less then 1 week) at low temperatures (-20 °C) before they were analysed to prevent phenolic degradation. Each extraction was performed in duplicate.

5.2.3.3 Determination of total phenolic content

The total phenolic content of the osmotic solution and gel extracts was determined spectrophotometrically by Folin-Ciocalteu's colorimetric method (Singleton & Rossi, 1965). TPH was expressed as the gallic acid equivalent (GAE) using the standard curve prepared at different concentrations of gallic acid. The data are presented as the average of two measurements for each extract.

5.2.3.4. Trolox Equivalent Antioxidant Capacity (TEAC) method

The antiradical scavenging capacity of gel extracts was assessed with the ABTS decolorization assay (Re et. al, 1999). This assay uses antioxidants to inhibit the absorbance of the radical cation of 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS), which has a characteristic long wavelength absorption spectrum with maxima at 734 nm. The extent of decolorization as a percentage of the inhibition of $ABTS^{+}\cdot$was determined as a function of concentration. It was calculated relative to the reactivity of Trolox, a water-soluble analog of vitamin E. By using the Trolox calibration curve, the antioxidant capacity of gel extracts was expressed as the Trolox equivalent antioxidant capacity, in millimoles per kilogram on a wet basis. Each determination was performed in duplicate.

5.2.3.5. HPLC analysis

The individual phenolics were identified and quantified with HPLC (Hewlett-Packard (HP/Agilent, Wardborn, Germany) equipped with ChemStation software. A Supelcosil column LC-18 (25 cm x 4.6 mm) with a particle size of 5 µm was used and an injection volume of 100µL was kept at 40 °C.

Phenolic compounds were eluted with a gradient elution of solvent A (glacial acetic acid in water at pH 2.60) and solvent B (20% solvent A and 80% of acetonitrile). The elution profile had the following proportions (v/v) of solvent A: 0-5 min, 100%; 5-10 min, 98%, 10-15 min, 96%; 15-30 min, 90%; 30-35 min, 80%; 35-40 min, 70%; 40-45 min 0%; 45-50 min, 100%. The flow rate of the mobile phase was kept constant at 1.5 mL/min, and the column temperature was kept at 40°C throughout the analysis. Peaks were monitored by an HPLC system equipped with a diode array detector (DAD), were identified by their retention times and UV-Vis spectra, and quantified using a calibration curve of the corresponding standard compound. A DAD was used to choose the maximum absorbance for each group of compounds, to control peak purity and to identify the spectra of some phenolics (Bétes-Saura et al, 1996).

Gallic acid (GA), protocatechuic acid (PA), (+)-catechin (CT), (-)-epicatechin (ECT), (-)-epicatechin 3-*O*-gallate (ECG), (-)-epigallocatechin 3-*O*-gallate (EGCG), (-)-epigallocatechin (EGC), procyanidin B1 (PAB1) and procyanidin B2 (PAB2) were purchased from Sigma-Aldrich (Steinheim, Germany). Results were expressed as milligrams of phenol per kilogram on a wet basis. All determinations were performed in duplicate.

5.2.4. Calculation procedures

The mass exchange between the solution and gel sample during OT was evaluated by using parameters such as water loss ($-\Delta M^{w}$), total soluble solid gain (ΔM^{ss}), sucrose gain (ΔM^{SUC}), NaCl gain (ΔM^{NaCl}), glycerol gain (ΔM^{GLY}), total phenolic gain (ΔM^{TPH}) and gain in moles of the osmo-active solute (ΔN^{ss}). These parameters were calculated using the following equations:

$$\Delta M^{j} = \frac{M_t \cdot x_t^j - M_0 \cdot x_0^j}{M_0} \qquad (5.1)$$

$$\Delta M^{SS} = \frac{M_t - M_0}{M_0} - \Delta M^{w} \qquad (5.2)$$

$$\Delta N^{ss} = \frac{M_t \cdot n_t^{ss} - M_0 \cdot n_0^{ss}}{M_0} \qquad (5.3)$$

where *M, x, and n* are the mass of the gel, and the mass and molar fraction of each component in the gel, respectively, the sub-indexes *0* and *t* indicate initial conditions and conditions at time t of treatment, and super-index *j* indicates each of the aforementioned components. From this point on, the mass fraction of each component in the gel will be expressed as kg/kg on a wet basis.

5.2.4.1. Determination of effective diffusion coefficients

The solution of Fick's second law for cubical configuration was used to model the mass transfer of water and solutes during OT. It was assumed that model food behaves like an isotropic structure, and that external resistance to mass transfer was negligible in comparison to the internal resistance. The initial and boundary conditions assumed that the cube's initial content of water and soluble solids was uniform, that the solid food was symmetrical with regard to the mass transfer direction, and that the model food surface was in thermodynamic equilibrium. The mass transfer was assumed to occur between the food liquid phase (i.e. food containing water and soluble components) and the osmotic solution. Therefore, the equilibrium criterion considered was that the food liquid phase and the osmotic solution are compositionally equal (Barat et al., 1998).

Under these assumptions, the total amount of each diffusing substance crossing the cube surface during a period of time *t* is given by Crank (1975):

$$Y_r^j = \frac{\left(z_t^j - z_\infty^j\right)}{\left(z^j - z_\infty^j\right)} = \left\{ \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 \frac{\pi^2 D_{ej}}{4a^2} t \right] \right\}^3 \qquad (5.4)$$

where Y_r^j indicates the ratio of each diffusing compound or group of compounds; z is the mass fraction in the food liquid phase; the subscripts 0,∞, and t represent initial concentrations, at equilibrium, and at any time; D_e is the effective diffusion coefficient, and a is half the thickness of the cube.

The values of D_e were determined by regressing experimental data to equation 5.4. The fitting was performed with SigmaPlot version 10.0 by applying the Marquardt-Levenberg algorithm. The regression coefficient, R^2, was used to measure how well the regression model described the data.

5.2.4.2. Determination of Peleg constants

The model proposed by Peleg (1988) and redefined by Palou et al. (1994) was employed to fit the progress of total and individual phenolic content during OT. Peleg's equation is an empirical model with two parameters initially established to describe sorption isotherms that approach equilibrium asymptotically:

$$\frac{t}{x_t^j - x_0^j} = k_1^j + k_2^j t \qquad (5.5)$$

where x is the mass fraction of each component expressed as kg/kg on a wet basis. Sub-indexes *0* and *t* indicate initial conditions and conditions at time t of treatment and super-index *j* indicates any of the components transferred. The constants k_1 and k_2 are the Peleg rate constant and Peleg capacity constant, respectively and are determined by fitting experimental data to equation 5.5. The Peleg rate constant, k_1, relates to the initial rate of mass change of any component:

$$\left.\frac{dx_t^j}{dt}\right|_{t=0} = \pm \frac{1}{k_1^j} \qquad (5.6)$$

The Peleg capacity constant k_2 relates to the contents at equilibrium, that is, the maximum phenolic content attainable after OT. As time $t \to \infty$, the equation (2) gives the relation between equilibrium (∞) conditions and k_2.

$$x_\infty^j = x_0^j \pm \frac{1}{k_2^j} \quad (5.7)$$

5.3. RESULTS AND DISCUSSION

5.3.1. Total phenolic content, phenolic profile, and antiradical scavenging capacity in the osmo-treated food

The total phenolic content in the osmo-treated food, determined by Folin-Ciocalteu's method, increased with processing time, as is shown in Figure 5.1. OT for 8 h with sodium chloride in the osmotic solution (OS) led to the highest total phenolic content in the osmo-treated food (5350 ± 65 mg of GAE/kg), followed by 5041 ± 35 mg of GAE/kg obtained after 8 h of OT with the control solution. When the sucrose-sodium chloride mixture, glycerol and sucrose were used as osmo-active solutes, the osmo-treated food showed a high but significantly lower total phenolic content, although the osmotic pressure of all the OSs was adjusted to obtain a constant a_w (0.935±0.010).

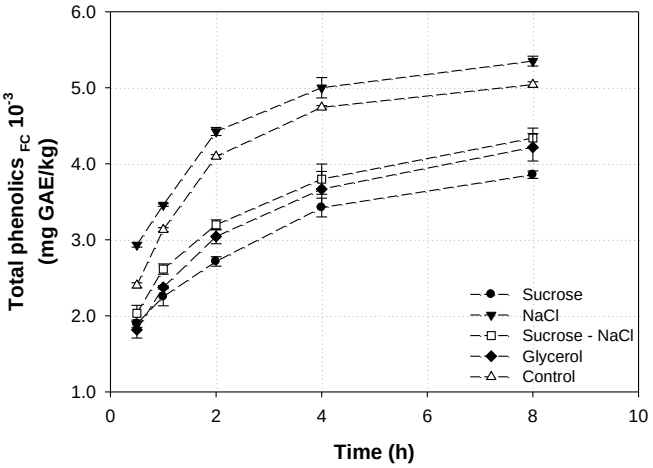

Figure 5.1. Total phenolic content in osmo-dehydrated food identified by Folin-Ciocalteu's method during OT.

Fruits such blueberries, cranberries, plums, strawberries and grapefruits have a total phenolic content around 2700-9300, 5272 ±215, 1740-3750, 1610-2900 and 1617 ± 76.6

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mg GAE/kg of FW, respectively, depending on the variety (Balasundram et al., 2006; Chun et al., 2005). Vegetables such broccoli, cabbage and carrot are also rich in phenolics which are found in concentrations of 875-1016, 546-925, 550-564 mg GAE/kg of FW, respectively (Balasundram et al., 2006).

A comparison of the total phenolic contents reported in the literature with those obtained for osmo-treated food showed that the total phenolic content of the model food was similar to or higher than that of the richest fruits and vegetables, even after a short processing OT time (1 h) with any of the osmo-active solutes tested.

The phenolic profile of the osmo-treated food is linked to the chemical composition of the grape seed extract used in the OS as a source of phenolics. Nine typical polyphenols of low molecular weight (Figure 5.2) from the phenolic groups of hydroxybenzoic acids, flavan-3-ol monomers and dimers were determined by HPLC.

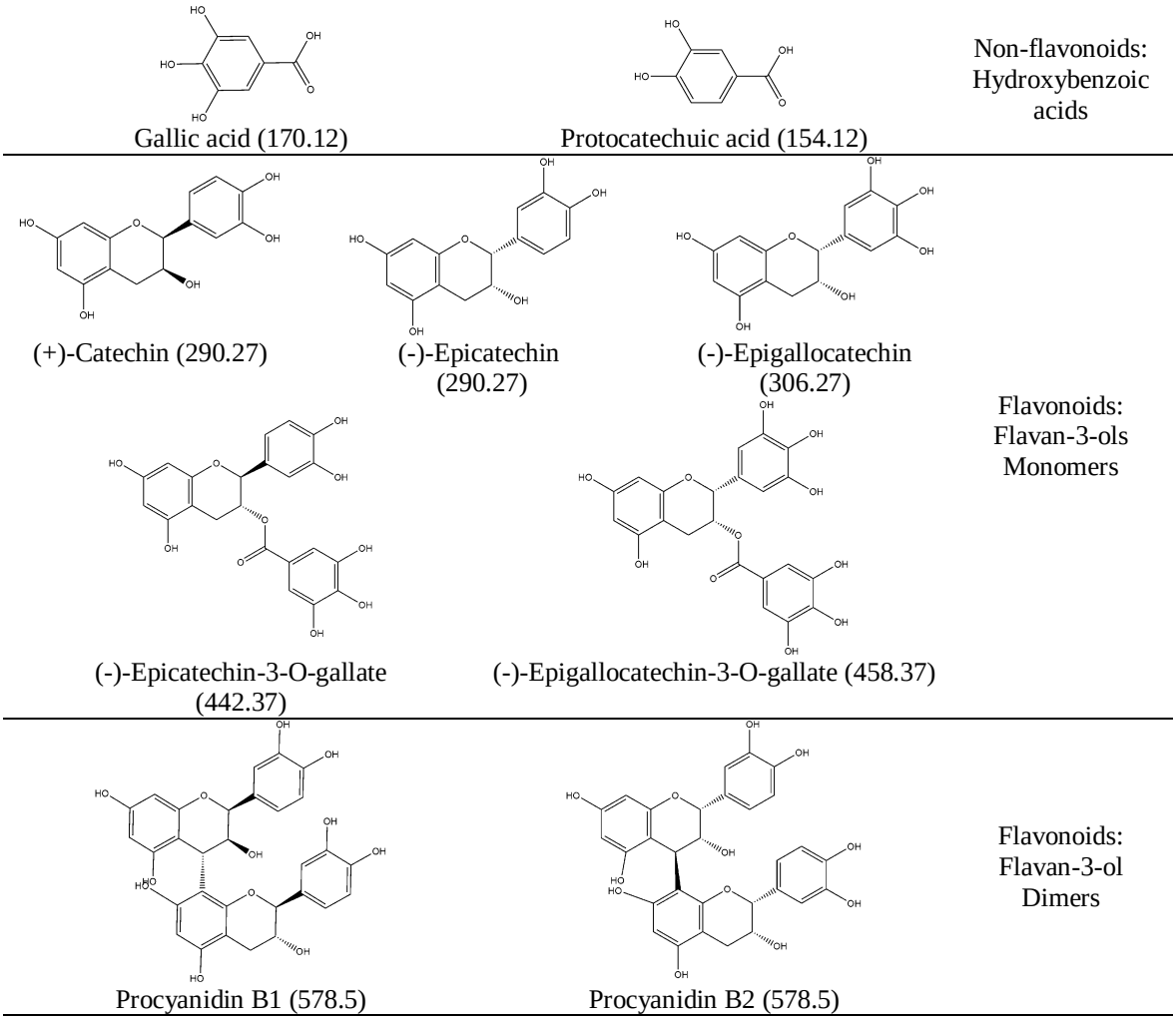

Figure 5.2. Molecular structure, corresponding molecular weight (in brackets) and classification of phenolic compounds identified in osmotic solution.

The analytical polyphenolic composition of grape seed extract is illustrated in Figure 5.3. Flavan-3-ol monomers were the major group quantified followed by flavan-3-ol dimers and hydroxybenzoic acids, which were 69%, 28% and 3%, respectively, of all individual phenolics quantified by HPLC. Moreover, the individual phenolics of low molecular weight determined were almost 59% of the total phenolics determined by Folin-Ciocalteu's method.

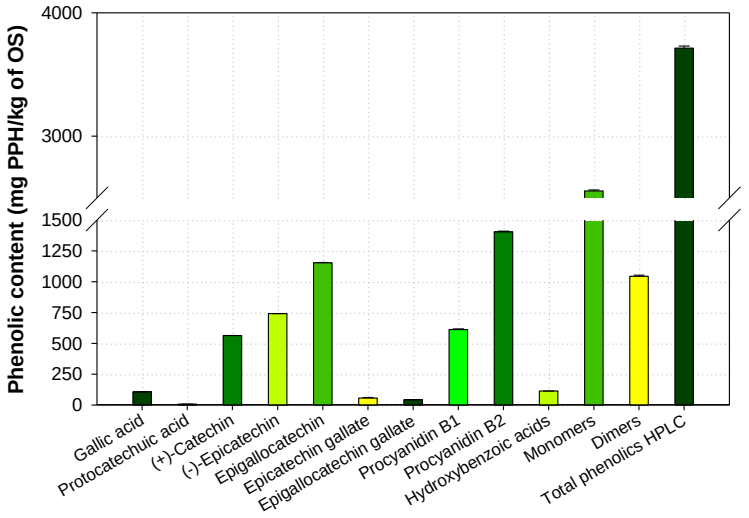

Figure 5.3. Phenolic composition of the osmotic solution (mean and standard deviation of determinations performed in triplicate).

For all of the conditions tested, the hydroxybenzoic acids, flavan-3-ol monomers and dimers present in the OS were also found in the osmo-treated food (Figure 5.4, 5.5 & 5.6). The influence of the osmo-active solute in OS on the gain in individual phenolics was the same as that observed in total phenolics.

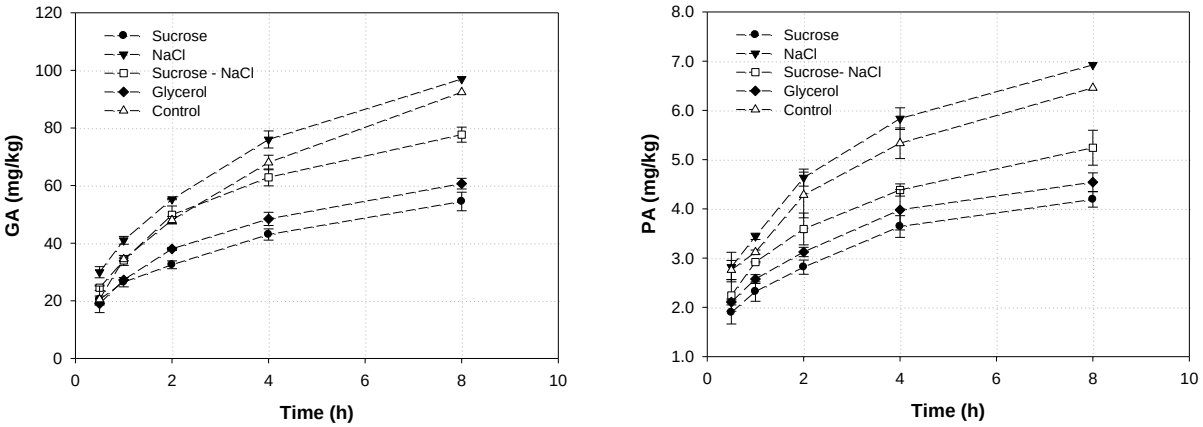

Figure 5.4. Content of non-flavonoids (hydroxybenzoic acids) identified in osmo-treated food during OT with different osmotic solutions (mean and standard deviation of experiments performed in duplicate).

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The concentration of individual phenolics was highest when sodium chloride was used as the osmotic agent. Of the phenolics identified and quantified in osmo-treated food the concentration was highest with flavan-3-ol monomers and dimers, since they were much more abundant in OS than hydroxybenzoic acids.

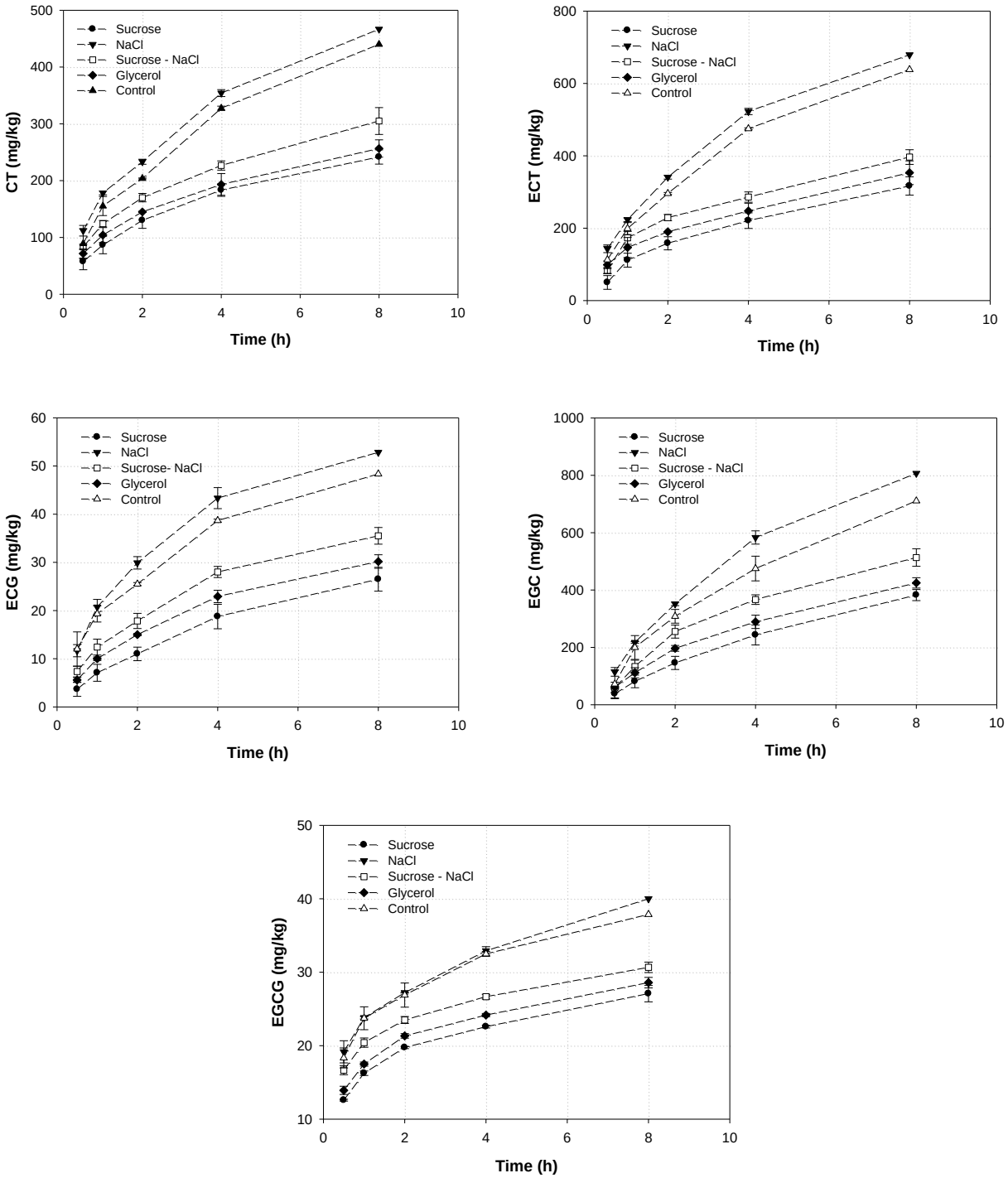

Figure 5.5. Content of flavan-3-ol monomers identified in osmo-treated food during OT (each plot represents OT with a different osmo-active solute in OS).

The concentrations of ECT and its gallate ester EGC in the osmo-treated food after 8 h of OT with an OS of sodium chloride were 679.2 ± 8.6 and 806.7 ± 23.1 mg GAE/kg, respectively. In this case, CT, PAB1 and PAB2 were found at concentrations of 466.7 ± 6.0, 488.3 ± 6.2 and 416.7 ± 27.2 mg GAE/kg, respectively. Of the flavan-3-ol monomers, ECG and EGCG were found at the lowest concentrations (52.8 ± 2.2 and 40.0 ± 0.6 mg GAE/kg, respectively).

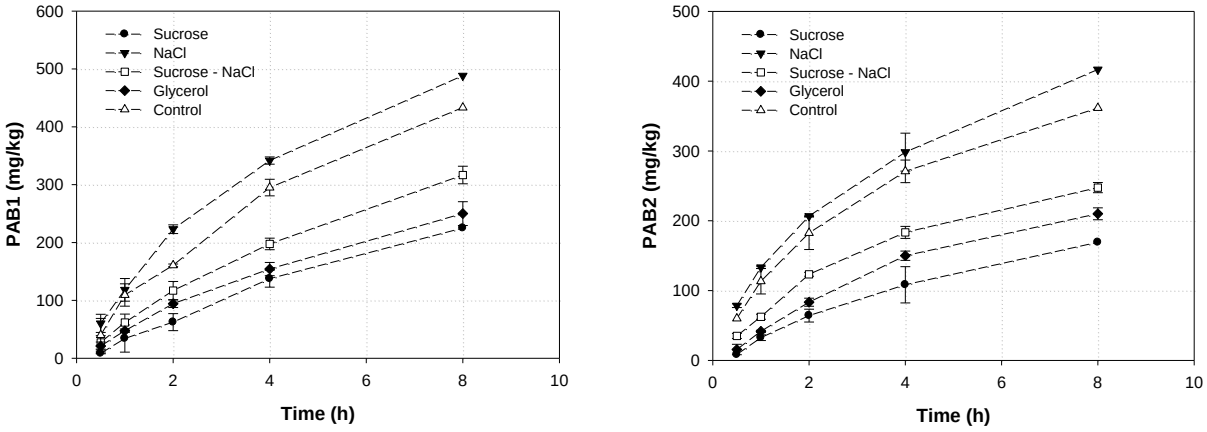

Figure 5.6. Content of flavan-3-ol dimers identified in osmo-treated food during OT with different osmotic solutions (mean and standard deviation of experiments performed in duplicate).

In the osmo-dehydrated food after 8 h of OT with an OS containing sodium chloride, GA was the hydroxybenzoic acid found at the highest concentration (97.0 ± 2.9 mg GAE/kg), followed by PA (6.9 ± 0.2 mg GAE/kg).

Concentrations of individual phenolics were lower when the sucrose-sodium chloride mixture, glycerol and sucrose were used as osmo-active agents. The total individual phenolic content obtained with the sucrose-sodium chloride mixture, glycerol and sucrose in OS was 68%, 57% and 51%, respectively, of all the phenolics identified when sodium chloride was the only osmo-active solute. According to these results, the type of osmo-active agent is a key parameter in the design of intermediate moisture products with a high phenolic infusion.

The antioxidant properties of the osmo-treated food (in particular, its antiradical scavenging capacity) were determined in vitro by TEAC. Although this assay is nonspecific and provides little information about the mechanisms controlling the antioxidant action, it is widely used to determine antioxidant capacity in foods, and it has

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provided a great deal of antioxidant data (Pellegrini et al 2003; Saura-Calixto & Goni, 2006).

Figure 5.7 shows the increase in TEAC of the model food during OT with different osmo-active solutes. As observed with total and individual phenolics, TEAC was highest when sodium chloride was used as an osmo-active agent.

a) b)

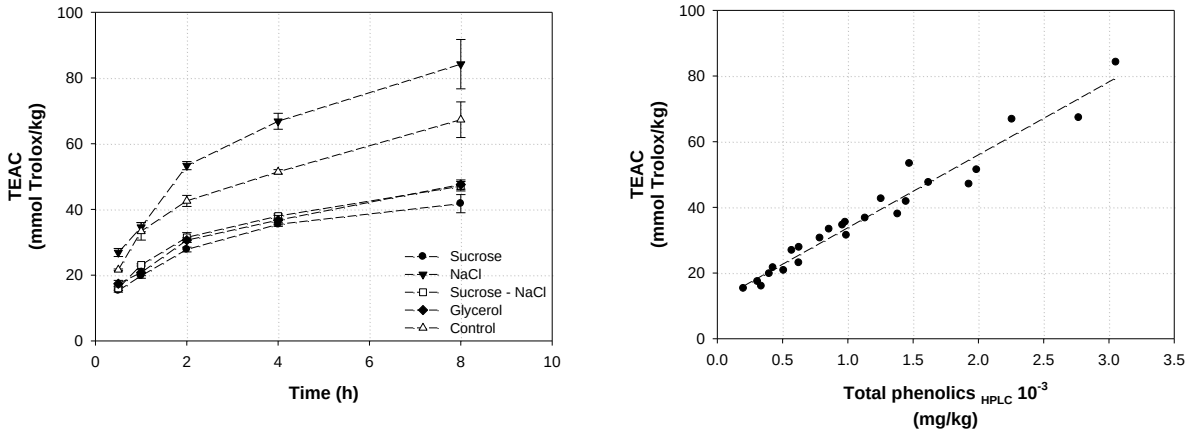

Figure 5.7. TEAC of (a) osmo-treated food during OT and (b) its linear relation with total phenolics determined with HPLC.

In these conditions and after 8 h of OT, TEAC was 84.2 ± 7.5 mmol of Trolox/kg. At the same processing time, the lowest antioxidant capacity (41.7 ± 2.8 mmol of Trolox/kg) was achieved using sucrose as the osmo-active solute. Even in these processing conditions an end product was obtained with a TEAC value that was between 2 - 3 times higher than that observed in fruits with the highest antioxidant capacity. For blackberry, raspberry and strawberry, TEAC values have been reported of 20.24, 16.79 and 10.94 mmol Trolox/kg FW, respectively (Pellegrini et al., 2003).

5.3.2. Mass transfer during OT: effect of the osmo-active solute

The results in Figure 5.8 show how the osmo-active agents in the OS and the immersion time affect the loss of water ($-\Delta M^{W}$) and mole solute gain (ΔN^{SS}) during OT of the model food. The nature of the solute was observed to have a notable influence on the values of $-\Delta M^{W}$ and ΔN^{SS} when different osmotic solutes were used. After 8h of OT, the highest $-\Delta M^{W}$ was 27.2 ± 0.4 % observed for OT with sucrose as the osmo-active solute, followed by 14.3 ± 0.08 % and 6.5 ± 0.0% for the sucrose-sodium chloride mixture and

glycerol, respectively. Experiments with sodium chloride led to a maximal water removal of 0.6 ± 0.03 % after 0.5 h treatment. From this point on, no more water was removed from the model food and the gel started to regain water.

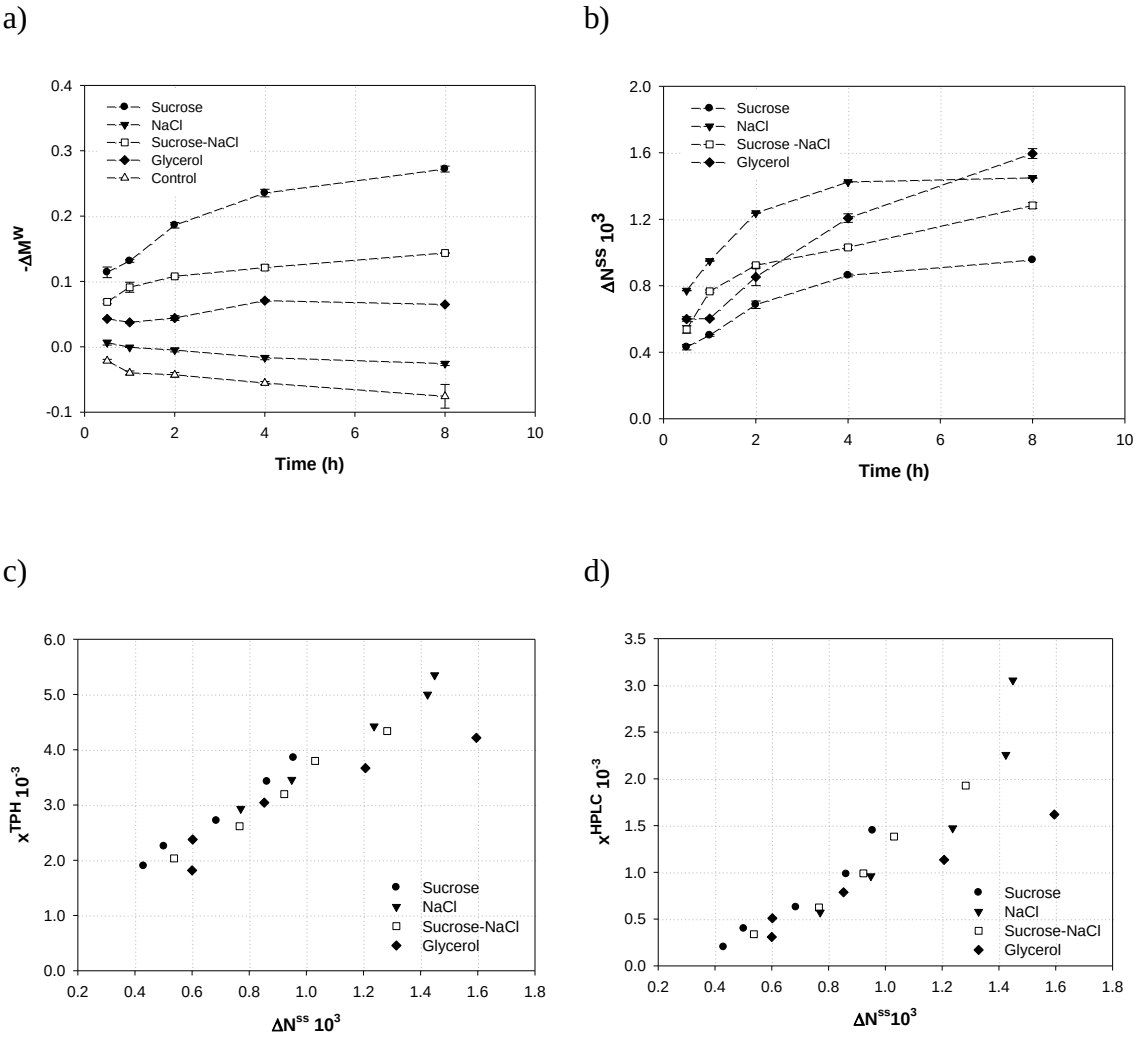

Figure 5.8. a) Mass changes of water ($-\Delta M^{w}$), b) gain of osmo-active solute, in moles (ΔN^{ss}), during OT, c) total phenolic content and d) phenolic content determined by HPLC versus the gain of osmo-active solute, in moles (mean and standard deviation of experiments performed in duplicate).

To better understand the effect of the molecular properties of osmo-active solutes on their infusion, we compared the mole gain during OT (Figure 5.8b) not the mass gain. For OSs with sodium chloride and sucrose as single osmo-active solutes, ΔN^{ss} varies from 0.08% to 0.14% and from 0.04% to 0.09% during the process, respectively. Although these results may be attributed to differences in the molecular weight of osmotic solutes, it is interesting to observe that after 8h of OT, ΔN of glycerol (MW(glycerol) = 92 g/mol) was higher (0.0016) than that of sodium chloride (0.0014,

MW(NaCl) = 58.4 g/mol). Figures 5.8c and 5.8d show how the total phenolic content determined by Folin-Ciocalteu's method and HPLC depend on mole solute gain. In all the cases, the phenolic content increases with solute gain, which indicates that the infusion of grape phenolics is coupled with the gain of osmo-active solutes. More precisely, it can be seen that sodium chloride in OS (either as a single osmo-active solute or in combination with sucrose) really promotes grape phenolic gain since a lower ΔN^{ss} than with glycerol OS led to the highest phenolic content.

Experimental data of phenolic mass transfer were fitted to the diffusional model (eq. 5.4) and Peleg's equation (eq. 5.5). To calculate the effective diffusion coefficients during OT with different osmo-active agents in the OS, the changes in moisture, soluble solids, and total and individual phenolics in the model food were calculated as dimensionless values related to the initial and equilibrium conditions. OT with the sucrose-sodium chloride mixture led to a moisture D_e = (2.84 10^{-10} m^2/s) higher than that obtained with sucrose (moisture D_e = 1.68 10^{-10} m^2/s) and glycerol (moisture D_e =7.38 10^{-11} m^2/s), respectively. In the experiments with sodium chloride as a single osmo-active agent in OS, the dewatering effect was so fast that after 0.5 h of OT no more water was removed from the model food and the gel started to regain water. In this situation the ratio of moisture does not fit the diffusional model. The transport rate of osmo-active solutes showed significant differences: the lowest D_e value was for glycerol (2.53 10^{-11} m^2/s) while the highest was for sodium chloride D_e either as a single osmo-active solute (3.49 10^{-10} m^2/s) or combined with sucrose (3.36 10^{-10} m^2/s).

As far as the mass transfer of grape phenolics is concerned, the D_e of total phenolics determined by Folin- Ciocalteu's method (Table 5.1) was lower than the moisture D_e for all the conditions tested in this study. The influence of the type of osmo-active solute on the mass transfer rate for total phenolics and hydroxybenzoic acids is similar: sodium chloride in OS led to the highest impregnation rates. The diffusional model, however, did not describe as precisely as Peleg's model the progress of total and individual phenolic content during OT as R^2 values show in Tables 5.1 and 5.2. More precisely, data related to flavan-3-ol monomers and dimers did fit poorly the diffusional model for any condition considered.

Considering this, values of Peleg's constants (Table 5.2) were used to quantify how the kind of osmo-active solute affects mass transfer of individual phenolics. The highest initial rate of phenolic mass transfer ($1/k_1$) was obtained with sodium chloride in OS for any of the individual phenolics analyzed. The non esterified flavan-3-ol monomers (CT;

ECT; EGC) showed a higher $1/k_1$ than their corresponding gallate esters (EGCG, ECG) with any of the osmo-active solutes considered. Besides, the progress of flavan-3-ol dimer (PAB1, PAB2) content did not fit the Peleg's model when sucrose, glycerol or no osmo-active solute was in OS. In any case $1/k_1$ of the flavan-3-ol dimers (PAB1, PAB2) was lower than that of the non esterified monomers.

To explain the effect of the osmo-active agent on phenolic mass transfer, the a_w gradient between the model food ($a_w food$ = 0.985) and the OS ($a_w solution$ = 0.935), which drives water transport during OT, was kept constant for all experiments. Other physical properties of OS, particularly viscosity, were investigated. Several authors have suggested that the viscosity of OS (Bohuon et al 1998, Cháfer et al 2001, Emam-Djomeh et al 2001) is the main factor affecting the mass transfer process during OT. Figure 5.9 plots the D_e and Peleg's constants of each individual phenolic against the kinematic viscosity of several OS at 20ºC. Viscosity data were obtained from the bibliography (Korosi et al., 1968; Wolf et al., 1984; Hugot et al., 1986; Shankar et al., 1994; Chenlo et al., 2002).

For each individual phenolic, the changes in D_e and Peleg's constants with the kinematic viscosity of the OS followed the same pattern. D_e and Peleg's constants dramatically decrease when the viscosity of OS increases from 1.1 10^{-6} (NaCl) to 2.32 10^{-6} (glycerol) m^2/s but it increases when viscosity reaches a value of 3.62 10^{-6} m^2/s (NaCl /sucrose). However, a higher increase in OS viscosity, up to 12.6 10^{-6} m2/s (sucrose), leads to a significant but less extensive decrease in D_e and Peleg's constants. In the experiments performed with an OS with no osmo-active agent (the control OS had the lowest kinematic viscosity), the values of grape phenolic D_e and Peleg's constants were lower than when an OS with sodium chloride was used. These results can be explained if we consider that when the control solution is used as the OS, phenolic infusion is controlled only by the gradient of the phenolic concentration between the solid food and the osmotic solution. As no significant differences were observed between the a_w of the model food and OS, nor a water out-flow either osmo-active solutes intake were occurring.

Viscosity, then, appears not to be the only physical property of OS which affects the mass transfer of grape phenolics during OT with a multi-component OS. Besides the a_w gradient between food and OS, the ionic nature of the osmo-active solute seems to promote phenolic infusion when a gel is used as a model food.

Table 5.1. Effective diffusion coefficients of moisture, and total and individual phenolics determined in osmo-treated food

	osmotic solution									
	sucrose		NaCl		sucrose - NaCl		glycerol		control	
	$D_e \times 10^{11}$ (m^2/s)	R^2	$D_e \times 10^{11}$ (m^2/s)	R^2	$D_e \times 10^{11}$ (m^2/s)	R^2	$D_e \times 10^{11}$ (m^2/s)	R^2	$D_e \times 10^{11}$ (m^2/s)	R^2
water	16. 8 ± 2.85	0.941	-	-	28.4 ± 5.03	0.952	7.38 ± 1.36	0.917	-	-
sucrose	16.8 ± 2.85	0.941	-	-	27.9 ± 4.99	0.952		-	-	-
NaCl		-	34.9 ± 6.44	0.949	33.6 ± 6.27	0.947		-	-	-
glycerol		-	-	-	-	-	2.53 ± 0.62	0.838	-	-
total phenolics $_{FC}$	7.52 ± 1.56	0.876	33.8 ± 6.40	0.944	10.9 ± 2.22	0.891	10.3 ± 1.93	0.909	25.6 ± 5.17	0.926
GA	3.59 ± 0.75	0.877	17.6 ± 3.38	0.944	9.42 ±1.57	0.939	5.08 ± 0.98	0.905	12.5 ± 2.75	0.923
PA	5.53 ± 1.13	0.878	25.8 ± 4.24	0.961	10.3 ± 1.86	0.916	7.77 ± 1.58	0.882	19.9 ± 3.18	0.955
CT	1.95 ± 0.60	0.786	11.2 ± 2.41	0.923	3.57 ± 0.78	0.881	2.51 ± 0.64	0.839	8.68 ± 2.03	0.905
ECT	1.74 ± 0.63	0.737	14.1 ± 3.78	0.901	3.42 ± 0.83	0.859	2.63 ± 0.65	0.847	10.7 ± 2.94	0.890
EGC	n.s. *	n.s.	5.84 ± 1.71	0.857	1.85 ± 0.73	n.s.	n.s.	n.s.	3.97 ± 1.26	0.825
EGCG	8.27 ± 1.72	0.878	32.1 ± 5.83	0.953	14.3 ± 3.39	0.855	11.2 ± 2.39	0.877	29.6 ± 5.23	0.950
ECG	2.02 ± 0.77	0.736	17.8 ± 4.47	0.918	4.97 ± 1.17	0.887	3.39 ± 0.91	0.849	13.3 ± 2.75	0.930
PAB1	n.s.	n.s.	7.87 ± 2.51	0.852	2.16 ± 0.93	0.706	n.s.	n.s.	5.26 ± 1.81	0.819
PAB2	n.s.	n.s.	0.72 ± 0.44	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
total phenolics $_{HPLC}$	n.s.	n.s.	5.40 ± 1.34	0.883	1.60 ± 0.59	0.721	1.06 ± 0.50	0.606	3.95 ± 1.10	0.851

*n.s. – not significant at $P<0.05$ confidence level

Table 5.2. Peleg constants of moisture, osmo-active solutes, total and individual phenolics determined during OT with several osmo-active agents.

	Sucrose			NaCl			Sucrose-NaCl			Glycerol			Control		
	$1/k_1 x10^8$ (s^{-1})	**$1/k_2 x10^5$ (g/g)**	**R^2**	**$1/k_1\ 10^8$ (s^{-1})**	**$1/k_2\ 10^5$ (g/g)**	**R^2**	**$1/k_1\ 10^8$ (s^{-1})**	**$1/k_2\ 10^5$ (g/g)**	**R^2**	**$1/k_1\ 10^8$ (s^{-1})**	**$1/k_2\ 10^5$ (g/g)**	**R^2**	**$1/k_1\ 10^8$ (s^{-1}))**	**$1/k_2\ 10^5$ (g/g)**	**R^2**
Total phenolics $_{FC}$	135.5±22.8	423.2±14.9	0.995	278.3±24.6	572.4±7.0	0.999	158.1±20.9	473.6±12.6	0.997	135.2±14.6	467.6±11.8	0.997	245.7±10.0	543.4±3.3	1.000
GA	1.19±0.24	6.28±0.44	0.980	1.73±0.24	11.7±0.74	0.984	1.57±0.145	9.23±0.34	0.995	1.21±0.13	7.19±0.30	0.993	1.23±0.12	12.12±0.76	0.984
PA	0.127±0.22	0.467±0.02	0.993	0.181±0.025	0.786±0.032	0.993	0.154±0.026	0.582±0.025	0.993	0.15±0.02	0.50±0.02	0.994	0.17±0.03	0.73±0.04	0.989
CT	3.36±0.28	31.5±1.62	0.989	6.45±0.78	60.6±4.6	0.977	4.88±0.67	37.6±2.67	0.980	4.19±0.54	31.56±2.07	0.983	5.19±0.56	60.80±5.17	0.972
ECT	3.38±0.33	45.7±4.00	0.970	8.29±0.543	93.8±4.7	0.990	5.81±0.81	50.3±4.09	0.974	5.51±1.10	43.03±4.51	0.957	6.69±0.37	94.33±4.91	0.989
EGC	2.37±0.08	87.6±7.25	0.973	6.96±0.19	135.2±4.7	0.995	4.12±0.39	93.5±13.6	0.920	3.68±0.12	69.74±2.93	0.993	5.19±0.62	137.13±29.24	0.840
EGCG	0.933±0.19	2.95±0.126	0.993	1.29±0.32	4.36±0.25	0.988	1.38±0.29	3.27±0.11	0.995	1.06±0.21	3.09±0.12	0.994	1.37±0.27	4.11±0.16	0.994
ECG	0.219±0.01	4.55±0.264	0.987	0.783±0.028	6.91±0.14	0.998	0.446±0.029	4.88±0.231	0.991	0.35±0.01	4.29±0.14	0.996	0.73±0.07	6.19±0.35	0.987
PAB1	n.s.*	n.s.	n.s.	3.81±0.15	89.4±5.59	0.985	1.74±0.10	89.0±16.9	0.870	1.35±0.09	72.73±16.61	0.820	n.s.	n.s.	n.s.
PAB2	n.s.	n.s.	n.s.	4.65±0.28	58.9±3.04	0.989	2.19±0.12	41.6±2.84	0.982	n.s.	n.s.	n.s.	1.23±0.12	53.58±0.90	0.999

*n.s. – not significant at P<0.05 confidence level

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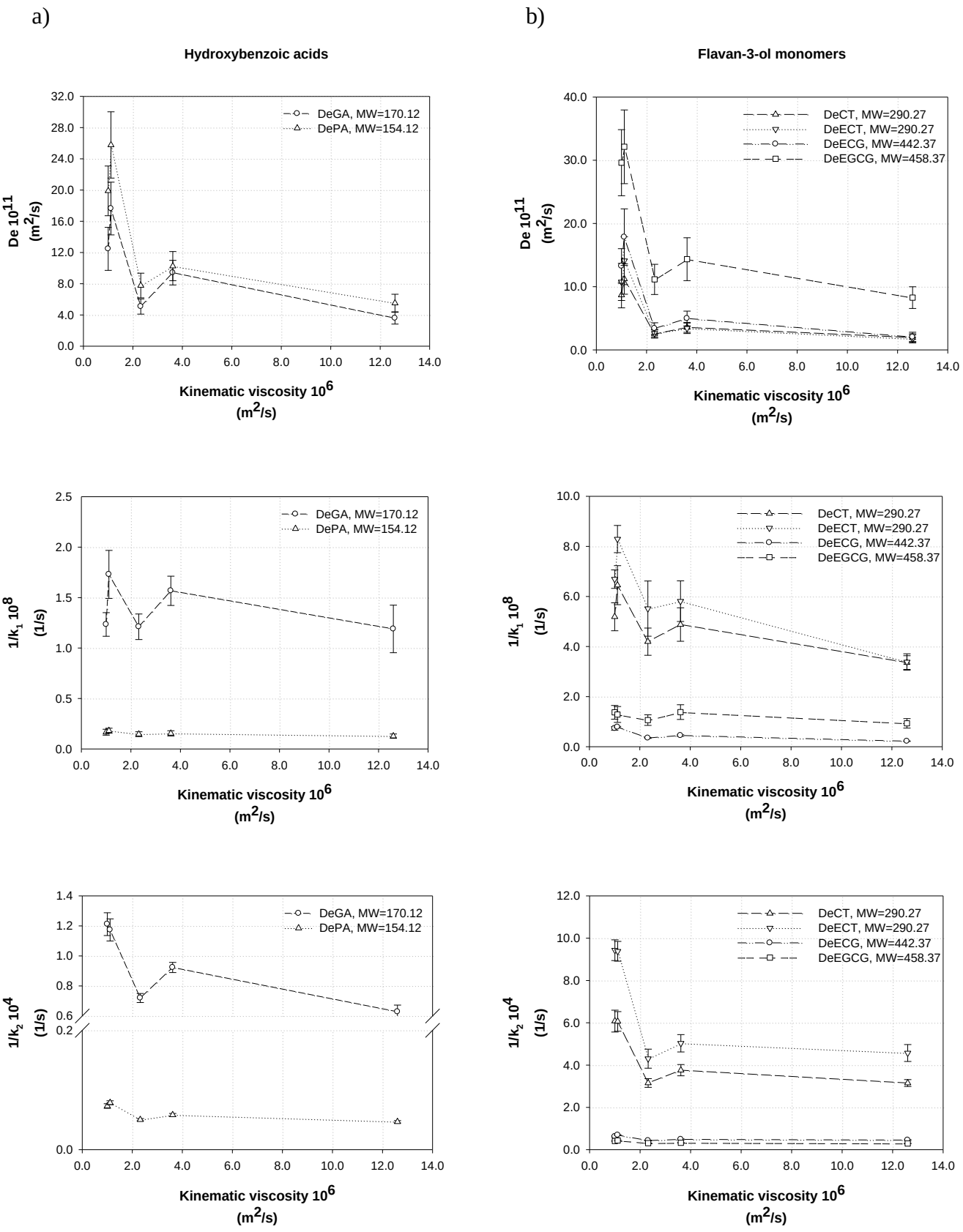

Figure 5.9. Diffusion coefficients and Peleg's constants of individual phenolics plotted against the kinematic viscosity of the osmotic solution: a) hydroxybenzoic acids and b) flavan-3-ol monomers.

5.4. CONCLUSIONS

Infusion of grape phenolics during OT with a multi-component OS made of osmo-active solutes and grape phenolics strongly depends not only on the a_w gradient between the food and OS but also on the kind of osmo-active solute used. Under the conditions used (total phenolic content = 6300 ± 45 mg GAE/kg and a_w = 0.935 ± 0.010 in OS), the infusion of grape phenolics was high with all the types of osmo-active solutes considered. In all the conditions tested, the total phenolic content of the osmo-treated food was similar to or even higher than that of the richest fruits and vegetables, so the antioxidant capacity of the product is also increased.

Of all the osmo-active solutes investigated, sodium chloride (either as a single osmo-active solute or in combination with sucrose) led to the highest phenolic infusion for each individual phenolic analyzed. This behavior could not be completely explained by differences in OS viscosity, so it seemed that ionic solutes promoted phenolic infusion during the OT of a model food gel. By selecting the OS composition, intermediate moisture products with a high content of phenolic compounds can be formulated.

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CHAPTER 6

*BINARY MIXTURES OF OSMO-ACTIVE SOLUTES (NaCl AND SUCROSE) AND THEIR EFFECT ON PHENOLIC PATTERN AND ANTIOXIDANT CAPACITY OF OSMO-TREATED PRODUCT**

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6.1. INTRODUCTION

The beneficial effect of grape phenolics on human health has been extensively reviewed and their recovery from by-products of winemaking and grape juice industry is thus a matter of growing interest. The typical by products of these industries are seeds, skins, and stems, all of which are rich sources of phenolics (particularly gallic acid and the flavans catechin and epicatechin). In addition, a wide variety of procyanidins, condensed phenolics containing the monomeric flavan-3-ol units of catechin and epicatechin, are found in grape seeds (Peng et al., 2001; Shi et al., 2003).

Recently, grape seed extracts (GSE) have become a widespread nutritional supplement because of their antioxidant properties. GSEs contain heterogeneous mixtures of monomers, oligomers, and polymers made up of sub-units of flavan-3-ol, and which depend on both the grape variety and the extraction conditions (Yilmaz et al., 2004; Gonzalez-Paramás et al., 2004). GSEs, and particularly those with high contents of oligomeric proanthocyanidins (dimer-, trimer- and tetrameric) have been shown to be highly bioavailable and to provide excellent health benefits: for example, a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress have been reported (Shi et al., 2003; Bagchi et al., 2000).

Osmotic treatment (OT), also called osmotic dehydration or dewatering impregnation soaking, is an operation that has a double effect: it partially dehydrates solid food material and simultaneously impregnates it with solutes. OT has been proposed as a method that will change a food formulation by: 1) reducing water content or adding water activity lowering agents, 2) supplementing the food with compounds that modify its functional and nutritional properties (Torreggiani et al., 2001).

The most common solutes in the osmotic solution are sodium chloride and sucrose, although other electrolytes and non-electrolytes, such us poly-alcohols and polysaccharides of different molecular weights (Spiess et al., 1998) have been used to control the ratio of dehydration to solute impregnation and optimize the end product formulation for each particular application. Generally, salts are used to treat vegetables, fish, meat and cheese, and sugars to treat fruits.

The use of multi-component solutions, basically ternary solutions of salt, sugar and water, lead to better control of water content and solute impregnation. OT with ternary solutions (NaCl/sucrose/water) has been applied to such fruits and vegetables as tomato (Tonon et al., 2007), apple (Sacchetti et al., 2001; Sereno et al., 2001), melon (Rodrigues

et al., 2007), carrot (Behsnilian et al., 2006), and paprika (Ade-Omowaye et al., 2002). In every case, water loss was increased and individual gain of each osmo-active solute reduced, which improved the sensory properties of the final food product (Sacchetti et al., 2001; Bohuon et al., 1998).

Even though OT has been extensively used to produce intermediate moisture products, it has only been used to a limited extent to produce functional foods from fruits and vegetables. In particular, it has been used to impregnate plant foods with probiotics (Alzamora et al., 2005) and minerals (e.g. calcium and zinc) (Martin-Diana et al., 2007). Recently, Rózek et al. (2007) described osmotic treatment as being a suitable method for infusing solid foodstuffs with grape phenolics. In this case, an agar-agar gel was used as a model food and a concentrated red grape must as the source of the phenolics. In all the conditions tested, low molecular weight phenolics (≤610 g/mol), and in particular *trans*-caftaric acid, *trans*-coutaric acid, ferulic acid, coumaric acid, caffeic acid (hydroxycinnamic acids), gallic acid (hydroxybenzoic acids), quercetin, and rutin (flavonols), were quantified in the osmo-dehydrated food. Under the conditions that maximized phenolic infusion, the total phenolic content of the gel was close to the values reported in some rich-in-phenolic fruits and vegetables while the Trolox Equivalent Antioxidant Capacity (TEAC) was three times that of fresh fruit with the highest antioxidant capacity. The concentration of the osmo-active solute, sucrose in that case, proved to control phenolic infusion and the antioxidant properties of the end product. However, the influence of the kind of osmo-active solute, used in single or binary mixtures, on the phenolic infusion pattern requires further research.

The main objective of this study is to investigate how the composition of the osmotic solution (the kind and concentration of the osmo-active agent) affected phenolic infusion, and the antioxidant properties and composition of the osmo-treated solid food. A phenolic-rich commercial GSE and an agar gel are used as a source of phenolics and a model food, respectively. Both osmo-active agents, NaCl and sucrose, are studied as single osmo-active solutes or in mixtures (NaCl and sucrose) with concentrations which maintain the same water activity (a_w).

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6.2. MATERIALS AND METHODS

6.2.1. Osmotic Solution and Model Food

A multi-component aqueous solution made of sucrose, NaCl and a commercial GSE (Vitisol® supplied by Berkem, Gardonne, France) was used as the osmotic solution. In all experiments the mass fraction of total phenolics was kept constant while the mass fraction of sucrose and NaCl was set by the experimental design described below.

The model food, an agar-agar gel, was prepared with 4% (w/w) agar-agar (Scharlau, Barcelona, Spain) and 9.6% (w/w) sucrose and distilled water. The mixture was heated to 95°C in a microwave oven until the agar-agar had completely dissolved. Gelation was achieved by cooling at room temperature. The gel was then stored at 6°±2°C prior to use within 2 days.

6.2.2. Osmotic treatment

The experimental set up consisted of two parts: a basket in which the gel samples were placed, and a vessel that was filled with the osmotic solution. The basket contained three shelves and guaranteed total immersion of the sample in the osmotic solution. About 50g of agar-agar gel cubes (1 cm side) was weighed and placed in the OT basket. This basket was then submerged in 1 L of osmotic solution. The model food was processed for 8 h, and the osmotic pressure of the solution was adjusted (see below). The solution/model food ratio (w/w) was always higher than 20:1 to prevent the solution from being significantly diluted by water removal, which would lead to local reduction of the osmotic driving force during the process. Temperature was maintained at 25 ±2°C. Agitation was provided by a magnetic stirrer. After osmotic treatment, the gel cubes were removed from the solution, gently blotted with tissue paper, and weighed. All experiments were run under atmospheric pressure.

6.2.3. Determination of Moisture Content

The moisture content of fresh and osmo-dehydrated food was determined with the 934.06 AOAC gravimetric method (1998).

6.2.4. Determination of Salt and Sucrose Content

About 2.5g of fine ground sample was dissolved in 50mL of miliQ water. Chloride content of osmo-dehydrated gel was determined by Mohr's method and expressed as a sodium chloride (James, 1995). The sucrose content of fresh and osmo-dehydrated was determined with a kit for sugar analysis based on a chemical method (Barceló, 1990) (GAB Sistemática Analítica S.L., Barcelona, Spain).

6.2.5. Extraction of Phenolic Compounds of the Osmo-treated Food

To determine the extent of phenolic impregnation in the osmo-treated food, a sequential extraction was carried out. About 5 g of homogenized sample was extracted sequentially with 30 mL of methanol: water (50/50, v/v) and 30 mL of acetone: water (50/50, v/v) for 1 h in each extraction solvent at room temperature. Each solvent extraction was carried out in duplicate. Filtrated and appropriately diluted extracts were taken for the determination of individual phenolic content and TEAC. Extracts (mixtures of water/methanol/acetone) used for HPLC analysis of individual phenolics were evaporated to dryness under reduced pressure (rotary evaporator, $T \leq 35°C$) and kept at –26ºC until analyzed, within 2 weeks. Then samples were re-suspended in high purity water (Milli-Q®, Millipore, Bedford, USA) and filtered through 0.45 µm syringe filters (TEKNOKROMA, Barcelona, Spain). In case of the osmotic solution, samples were filtered through 0.45 µm syringe filters and directly analyzed by HPLC.

6.2.6. Determination of Total Phenolic Content

The total phenolic content of the osmotic solution was determined with Folin-Ciocalteu's method (Singleton et al., 1965). The results were expressed as gallic acid equivalents (mg of GAE/kg of wet basis). The total phenolic content of the treated gel was determined as the sum of all phenolics as detected by HPLC analysis.

6.2.7. Determination of Individual Phenolics by HPLC

Phenolics were identified and quantified by HPLC (Hewlett-Packard (HP)/Agilent, Wardborn, Germany). An automatic injector, HP 1000, was used for the injection. A Supelcosil LC-18 column (25 cm x 4.6 mm), with a particle size of 5µm and an injection volume of 100µL was kept at 40°C.

A constant flow rate of 1.5 mL/min was used with two solvents: solvent A, glacial acetic acid in water to adjust pH to 2.60; and solvent B, 20% solvent A with 80% of

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acetonitrile. The composition of the mobile phase during the analysis was set according to the elution program explained by Betés-Saura et al. (1996). Peaks were monitored by an HPLC system equipped with a diode array and were identified by their retention times and spectra with external standards. A diode array UV-vis detector (DAD) was used to choose the maximum absorbance for each group of compounds, to control peak purity, and to identify the spectra of some phenolics (Betés-Saura et al., 1996). The concentrations of the phenolic compounds identified were measured using external standard curves. Calibration curves (standard area in absorbance versus concentration in mg/L) were performed over the range of concentration observed.

Gallic acid (GA), protocatechuic acid (PA), (+)-catechin (CT), (-)-epicatechin (ECT), (-)-epicatechin 3-*O*-gallate (ECG), (-)-epigallocatechin 3-*O*-gallate (EGCG), (-)-epigallocatechin (EGC), procyanidin B1 (PAB1) and procyanidin B2 (PAB2) purchased from Sigma-Aldrich (Steinheim, Germany) were used as standards for the identification and quantification of individual phenolics. Results were expressed as miligrams of phenol per kilogram on a wet basis.

6.2.8. Trolox Equivalent Antioxidant Capacity (TEAC)

The antioxidant capacity of the osmo-treated material extracts obtained as described above was determined as Trolox Equivalent. The method compares the ability of antioxidant molecules to quench the long-lived $ABTS^{\cdot+}$, a blue-green chromophore with characteristic absorption at 734 nm, with that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble vitamin E analogue (Re et al., 1998).

All determinations were carried out in duplicate. The percentage inhibition was compared with the standard calibration curve for Trolox (R^2=0.999), and the results were expressed as the Trolox equivalent in milimoles per kilogram on a wet basis.

6.2.9. Experimental Design

A second-order Central Composite Rotatable Design (CCRD) with two factors (NaCl and sucrose molality of the osmotic solution) was used to determine their effect on the response pattern. The experimental design consisted of three sets of points: the 2^k vertices ($\pm 1, \pm 1$) of a *k*-dimensional 'cube', the 2^k vertices ($\pm\alpha, 0; 0, \pm\alpha$) of an axial or 'star' at distance α from the centre of the design, and a number, n_0, of 'center points' for two factors ($k = 2$). Five levels of each actual variable were considered following the

rotatability criterion (the values required were α = 1.414) and the central point was replicated six times according to Khuri and Cornell (Khuri et al., 1996). The actual factor values and the corresponding coded values are given in Table 6.1.

Table 6.1 The actual factor values (NaCl and sucrose molality of the osmotic solution) and the corresponding coded values

Osmotic Solution				
	Actual factor values		Coded values	
	Sucrose (molality)	NaCl (molality)	Sucrose (molality)	NaCl (molality)
Nr.	X_1	X_2	X_1	X_2
1	2.49	1.62	1	1
2	0.43	1.62	-1	1
3	2.49	0.28	1	-1
4	0.43	0.28	-1	-1
5	1.46	0.95	0	0
6	1.46	0.95	0	0
7	1.46	0.95	0	0
8	0	0.95	-1.414	0
9	1.46	0	0	-1.414
10	2.92	0.95	1.414	0
11	1.46	1.9	0	1.414
12	1.46	0.95	0	0
13	1.46	0.95	0	0
14	1.46	0.95	0	0

The molality of the two osmo-active solutes (sucrose and NaCl) were the actual factors selected to adjust the osmotic strength and, therefore, the a_w of the osmotic solution. In OT, the solid foodstuff loses water while the a_w difference between the solid food and the osmotic solution is significant. The maximum values of the actual factors were those leading to a water activity of 0.935 while the minimum value considered for them both was zero. Previous results showed that phenolic impregnation was significant and water loss considerable in OT with a concentrated grape juice of 0.935 a_w (Rózek et al., 2007).

6.2.10. Statistical Analysis

Variance analysis, calculation of regression coefficients, and tridimensional graphics were performed using SigmaPlot 9.0 and SPSS 13.0 programs.

6.2.11. Calculation Procedures

The mass transfer of the model food during OT was evaluated by calculating the water loss ($-\Delta M^{w}$), total solid gain (ΔM^{SS}), sucrose gain (ΔM^{SUC}), NaCl gain (ΔM^{NaCl}), and phenolic gain (ΔM^{TPH}). These parameters were calculated as:

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$$\Delta M^{w} = \frac{M_t \cdot x_t^{w} - M_0 \cdot x_0^{w}}{M_0} \quad (6.1)$$

$$\Delta M^{SS} = \frac{M_t - M_0}{M_0} - \Delta M^{w} \quad (6.2)$$

$$\Delta M^{SUC} = \frac{M_t \cdot x_t^{SUC} - M_0 \cdot x_0^{SUC}}{M_0} \quad (6.3)$$

$$\Delta M^{NaCl} = \frac{M_t \cdot x_t^{NaCl} - M_0 \cdot x_0^{NaCl}}{M_0} \quad (6.4)$$

$$\Delta M^{TPH} = \frac{M_t \cdot x_t^{TPH} - M_0 \cdot x_0^{TPH}}{M_0} \quad (6.5)$$

where *M* and *x* are the mass of the gel and the mass fraction of each component in the gel, respectively, the sub-indexes *0* and *t* indicate initial conditions and conditions at time t of treatment, and super-indexes *w, SS, SUC, NaCl* and *TPH* are water, total solids, sucrose, sodium chloride, and total phenolics, respectively. The total phenolic content of the treated gel was determined as the sum of all phenolics as detected by the HPLC analysis. From this point on, the mass fraction of each component in the gel will be expressed as kg/kg on a wet basis.

In order to establish how the kind of osmo-active solute affected the response variables (gain and mass fraction of sucrose, sodium chloride, phenolics, TEAC, etc. in the osmo-treated food), we calculated the response values from the second-order model for those combinations of actual factors that led to an osmotic solution of 0.935 a_w. By applying the Norrish (Norrish, 1996) and Pitzer (Pitzer, 1973) equations for predicting the a_w of binary solutions of sucrose and NaCl, respectively, and the Ross equation for ternary solutions (Ross, 1975), we calculated the concentrations of sucrose and NaCl required to obtain several aqueous ternary solutions with a common a_w value of 0.935.

6.3. RESULTS AND DISCUSSION

6.3.1. Phenolic Profile of the Osmotic Solution

A commercial GSE was used as a source of phenolics and all experiments were performed with a total phenolic concentration of 6300±45 mg GAE/kg in the osmotic solution. Table 6.2 shows the total and individual phenolics quantified by Folin-Ciocalteau's method and HPLC, respectively, their molecular weight and structure, and classification. Phenolics of low molecular weight were identified and quantified (< 600 g/mol) since they were found to be the ones that infused most in solid food during OT (Rózek et al., 2007). Of the individual phenolics, flavan-3-ol monomers were the major group quantified followed by flavan-3-ol dimers and hydroxybenzoic acids, which represented 69%, 28%, and 3%, respectively, of all the individual phenolics identified by HPLC. In addition, the individual phenolics of low molecular weight quantified were almost 59% of the total phenolics determined by Folin-Ciocalteu's method.

Of the flavan-3-ol monomers, EGC was found at the highest level (1153.4±2.7 mg/kg) followed by ECT (741.0±0.2 mg/kg) and CT (563.0±0.5 mg/kg). The gallate esters, ECG and EGCG, were present in lower concentrations (56.5±4.1 and 42.4±0.3 mg/kg, respectively) than the corresponding ECT and EGC. PAB1 was the flavan-3-ol dimer detected in the highest concentration (611.4±5.8 mg/kg) followed by PAB2 (432.1±5.55 mg/kg) while GA and PA were the hydroxybenzoic acids found at average levels of 106.0±0.4 and 7.4±0.1 mg/kg, respectively.

The GSEs of different grape cultivars have been widely characterized (Guendez et al., 2005; Kammemer et al., 2004) and their profile of low molecular weight phenolics was similar to the one determined in the osmotic solution. CT and ECT are usually the major catechins and PAB1 and PAB2 the most abundant procyanidin dimers. However, grape variety, maturation stage, cultural practices and extraction conditions may cause significant differences in the particular phenolic profile of each GSE (Rodriguez-Montealegre et al., 2006).

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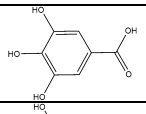
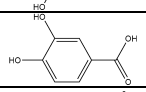
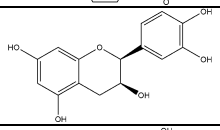
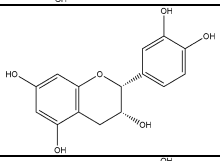
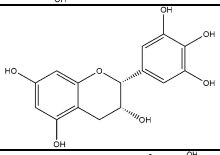
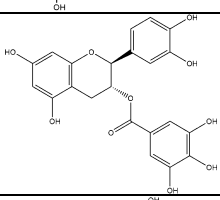
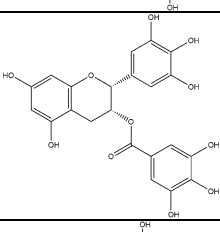
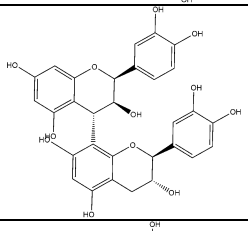
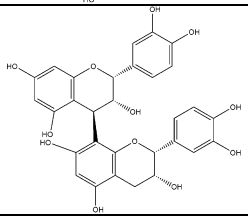
Table 6.2. Phenolic composition of the osmotic solution (mean ± standard deviation of determinations performed in triplicate)

	Concentration [mg/kg]	Molecular weight [g/mol]	Molecular structure	Phenolic classification
Gallic acid (GA)	106.0± 0.4	170.12		Non-flavonoids: Hydroxybenzoic acids
Protocatechuic acid (PA)	7.4±0.1	154.12		
(+)-Catechin (CT)	563.0±0.5	290.27		Flavonoids: Flavan-3-ol Monomers
(-)-Epicatechin (ECT)	741.0±0.2	290.27		
(-)-Epigallocatechin (EGC)	1153.4±2.7	306.27		
(-)-Epicatechin 3-O-gallate (ECG)	56.5±4.1	442.37		
(-)-Epigallocatechin 3-O-gallate (EGCG)	42.4±0.3	458.37		
Procyanidin B1 (PAB1)	611.4±5.8	578.5		Flavonoids: Flavan-3-ol Dimers
Procyanidin B2 (PAB2)	432.1±3.56	578.5		
Total phenolics$_{FC}$ [a]	6300±45.0			
Total phenolics$_{HPLC}$ [b]	3713.3±17.6			
Hydroxybenzoic acids	113.5 ±0.4			
Monomers	2556.3±7.8			
Dimers	1043.5±9.3			

[a. b] Total phenolics determined using the Folin-Ciocalteau method and HPLC, respectively. Total phenolics$_{FC}$ is expressed as mg GAE/kg.

Several responses were analyzed: those related to mass transfer during OT (ΔM of water, total solids, sucrose, NaCl, and phenolics), the composition of the osmo-treated food (mass fraction of sucrose, NaCl, and individual phenolics), and its anti-radical scavenging capacity (TEAC). Tables 6.3 and 6.4 show the results of the experimental design for all the responses analyzed. Tables 6.5 and 6.6 show the linear, quadratic and interaction effects of the second order polynomial model (coefficient value and significance level). Standard errors (SE) and the t- and P-values of the regression coefficients are also shown.

The response surfaces computed using only the significant effects at the $P<0.05$ level are shown in Figures 6.1 and 6.2. ΔM^{TPH} was significant throughout the response surface (from 0.17±0.05 % to 0.27±0.03 %) corresponding to ΔM^{NaCl} and ΔM^{SUC} values between 0 and 2.3±0.1 %, and 7.4±2.6% and 36.0±3.0 %, respectively.

Negative values of ΔM^{SUC} indicate that low sucrose concentrations in the osmotic solution wash out the sucrose initially present in the model food (8.6%, w/w). Throughout the range studied, the significant gains in phenolics corresponded with a different extent of water loss: $-\Delta M^{w}$ was between 0 and 27±3%. Overall, the ratio of water loss to phenolic gain, $-\Delta M^{w}/\Delta M^{TPH}$, was mainly controlled by the sucrose concentration of the osmotic solution, and reached a maximum value of about 149 when the sucrose concentration was the highest. Figure 6.2 shows the model food composition after 8 hours of OT versus the concentration of NaCl and sucrose in the osmotic solution. Under these conditions, an intermediate moisture product was obtained with a_w values between 0.912 and 0.955 (Table 6.3).

Even though the osmo-treated food presents quite a narrow range of a_w, its composition depended heavily on the concentration of NaCl and sucrose in the osmotic solution. When the composition of the osmotic solution was varied, the NaCl content in the osmo-treated food ranged between 0 and 0.079±0.002 kg/kg and sucrose levels between 0.03±0.01 and 0.427±0.024 kg/kg.

Table 6.3. Experimental design and water loss ($-\Delta M^{W}$), total solid ($-\Delta M^{SS}$), sucrose ($-\Delta M^{SUC}$), and NaCl gain ($-\Delta M^{NaCl}$), sucrose and NaCl content, water activity, total phenolics determined by HPLC, and TEAC of the osmo-treated gel at the different factor levels. OT was performed for 8 hours.

Osmotic Solution		Osmo-treated food								
Sucrose (molality)	NaCl (molality)	ΔM^{W}	ΔM^{SS}	ΔM^{SUC}	ΔM^{NaCl}	Sucrose	NaCl x 10^3	a_w	TPH_{HPLC}	TEAC
X_1*	X_2*					(kg/kg)	(kg/kg)		(mg/kg)	(mmolTrolox/kg)
2.49 (1)	1.62 (1)	-0.234	0.340	0.334	0.049	0.394 ±0.004	44.3 ±0.2	0.941	1598.8 ±39.0	42.1 ±1.2
0.43 (-1)	1.62 (1)	-0.033	0.072	0.044	0.065	0.141 ±0.002	62.5 ±0.2	0.931	2177.2 ±93.4	68.4 ±5.2
2.49 (1)	0.28 (-1)	-0.206	0.300	0.336	0.009	0.400 ±0.004	8.5 ±0.1	0.936	1660.6 ±177.6	41.4 ±0.9
0.43 (-1)	0.28 (-1)	-0.011	0.012	0.040	0.012	0.142 ±0.001	11.9 ±0.2	0.935	2427.6 ±49.1	64.1 ±4.6
1.46 (0)	0.95 (0)	-0.143	0.228	0.231	0.035	0.307 ±0.003	32.4 ±0.4	0.938	1852.1 ±74.0	48.2 ±0.3
1.46 (0)	0.95 (0)	-0.144	0.229	0.237	0.035	0.313 ±0.003	32.4 ±0.8	0.938	1848.5 ±66.1	45.5 ±5.1
1.46 (0)	0.95 (0)	-0.143	0.229	0.229	0.036	0.305 ±0.002	32.8 ±0.2	0.942	2208.1 ±34.1	47.5 ±1.1
0 (-1.414)	0.95 (0)	0.049	-0.067	-0.087	0.042	0.016 ±0.000	42.7 ±0.2	0.935	2997.2 ±139.3	65.7 ±6.7
1.46 (0)	0 (-1.414)	-0.102	0.186	0.241	0.000	0.316 ±0.002	0	0.955	1958.8 ±100.3	45.0 ±2.6
2.92 (1.414)	0.95 (0)	-0.271	0.323	0.329	0.027	0.410 ±0.003	25.9 ±0.1	0.932	1893.9 ±80.4	37.7 ±0.6
1.46 (0)	1.9 (1.414)	-0.143	0.237	0.210	0.067	0.285 ±0.001	61.7 ±0.1	0.912	2112.5 ±158.3	47.7 ±2.1
1.46 (0)	0.95 (0)	-0.132	0.211	0.214	0.035	0.29 3±0.001	32.2 ±0.1	0.942	1881.9 ±80.6	44.4 ±0.6
1.46 (0)	0.95 (0)	-0.149	0.211	0.212	0.034	0.296 ±0.001	32.1 ±0.2	0.938	1816.5 ±117.2	46.3 ±0.9
1.46 (0)	0.95 (0)	-0.134	0.215	0.203	0.035	0.282 ±0.001	31.9 ±0.1	0.940	1772.7 ±91.5	45.1 ±0.9

(*) In brackets, the coded values of the two factors.

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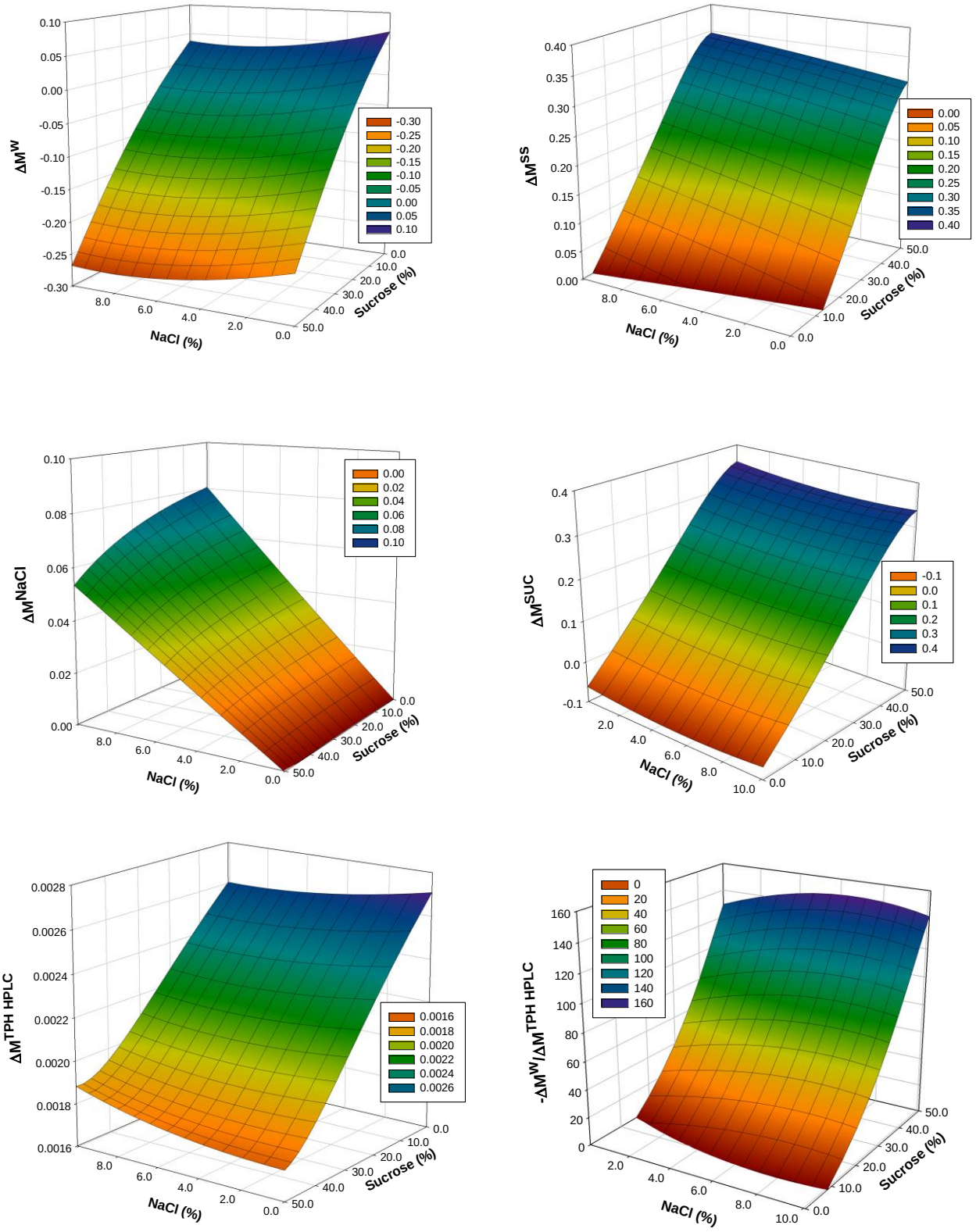

Figure 6.1. Surface responses of water mass changes (ΔM^{w}), gain in total solids (ΔM^{ss}), NaCl (ΔM^{NaCl}), sucrose (ΔM^{SUC}), total phenolics (ΔM^{TPH}) and ratio of water loss to gain in total phenolics ($\Delta M^{w}/\Delta M^{TPH}$) versus sucrose and NaCl mass fraction in the osmotic solution.

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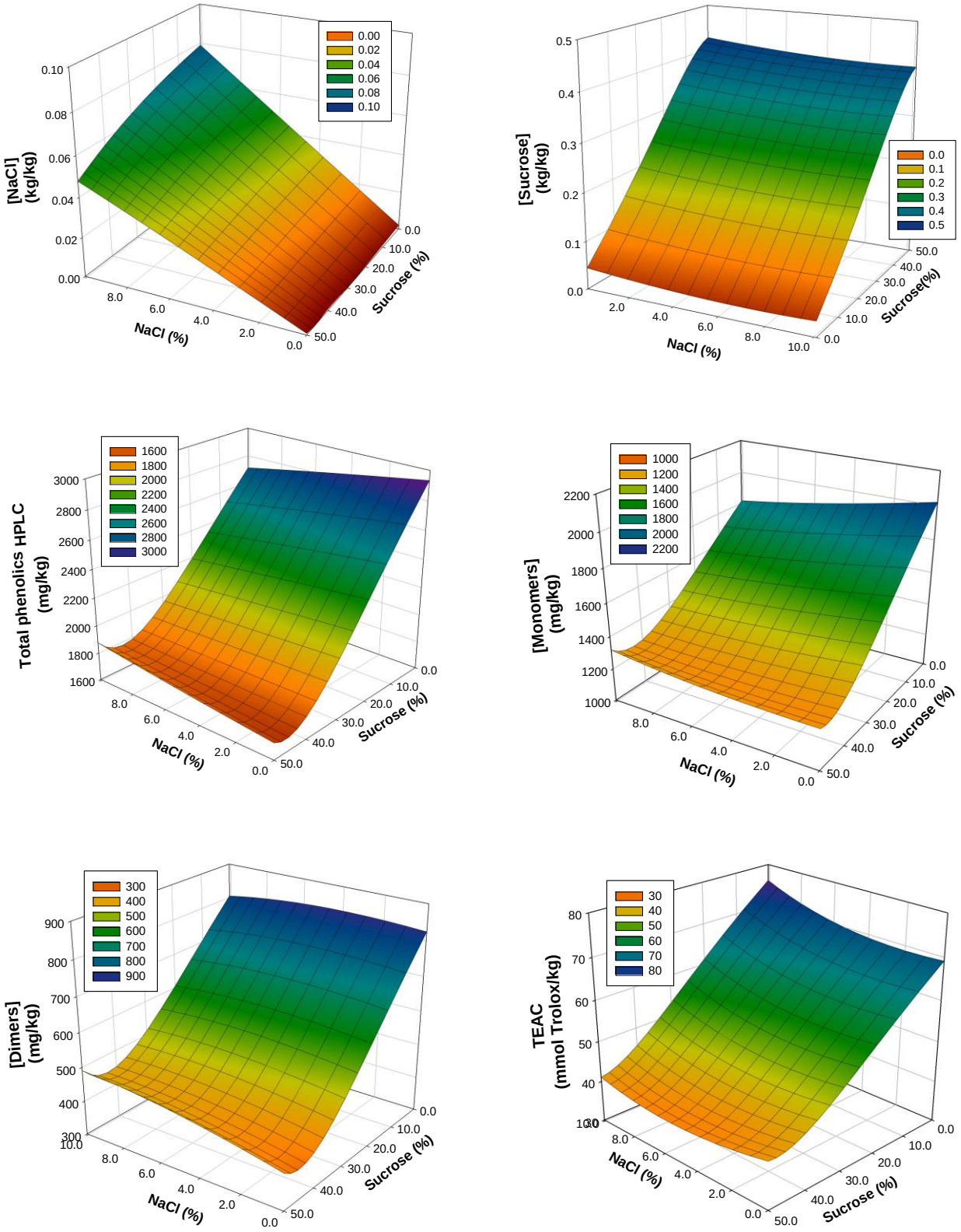

Figure 6.2. Surface responses of NaCl, sucrose, total phenolic, flavan-3-ol monomer and dimer content, and TEAC of the osmo-treated food after 8h of OT.

Table 6.4. Experimental design and content of individual phenolics determined by HPLC in the osmo-treated gel at the different factor levels. OT was performed for 8 hours

Osmotic Solution		Osmo-treated food								
Sucrose (molality) X_1	NaCl (molality) X_2	GA (mg/kg)	PA (mg/kg)	CT (mg/kg)	ECT (mg/kg)	EGC (mg/kg)	ECG (mg/kg)	EGCG (mg/kg)	PAB1 (mg/kg)	PAB2 (mg/kg)
2.49 (1)	1.62 (1)	54.7 ± 0.1	6.6 ± 0.0	272.5 ± 3.3	354.1 ± 4.3	448.6 ± 25.5	30.8 ± 0.5	27.0 ± 0.0	265.3 ± 0.6	139.1± 4.8
0.43 (-1)	1.62 (1)	68.7 ± 0.1	6.5 ± 0.1	357.6 ± 4.9	467.2 ± 7.6	575.2 ± 45.6	51.0 ± 1.7	30.3 ± 0.6	415.3 ± 5.8	205.4 ± 26.9
2.49 (1)	0.28 (-1)	63.9 ± 0.1	5.8 ± 0.4	298.9 ± 15.1	387.4 ± 20.3	458.1 ± 90.8	33.3 ± 1.4	27.5 ± 0.5	244.9 ± 45.7	140.9 ± 3.3
0.43 (-1)	0.28 (-1)	75.1 ± 0.8	8.1 ± 1.7	390.9 ± 1.8	513.0 ± 5.6	704.1 ± 37.5	51.8 ± 0.5	31.1 ± 0.0	445.2 ± 0.9	208.2 ± 0.2
1.46 (0)	0.95 (0)	65.4 ± 0.2	6.6 ± 0.6	321.6 ± 5.3	415.6 ± 9.4	517.0 ± 44.7	38.1 ± 0.6	27.6 ± 0.1	309.6 ± 6.5	150.5 ± 6.5
1.46 (0)	0.95 (0)	62.0 ± 0.0	7.1 ± 1.4	311.6 ± 4.3	406.0 ± 5.4	513.9 ± 36.8	36.8 ± 0.3	27.8 ± 0.2	317.2 ± 7.7	166.1 ± 10.0
1.46 (0)	0.95 (0)	74.6 ± 0.3	6.9 ± 0.3	371.6 ± 1.8	482.3 ± 2.3	625.6 ± 22.5	43.2 ± 0.4	29.6 ± 0.1	385.2 ± 2.4	189.3 ± 4.1
0 (-1.414)	0.95 (0)	86.9 ± 0.1	6.9 ± 0.1	457.0 ± 11.9	617.8 ± 16.5	836.4 ± 77.0	71.3 ± 4.0	34.7 ± 0.5	598.9 ± 18.1	287.3 ± 11.2
1.46 (0)	0 (-1.414)	62.4 ± 0.7	5.0 ± 0.1	345.1 ± 5.7	448.1 ± 8.0	553.3 ± 42.0	41.2 ± 0.5	29.0 ± 0.2	317.5 ± 31.4	157.2 ± 11.9
2.92 (1.414)	0.95 (0)	65.5 ± 0.1	5.8 ± 0.1	319.6 ± 3.4	417.0 ± 6.1	528.5 ± 39.2	35.0 ± 0.1	28.6 ± 0.2	313.7 ± 12.3	180.1 ± 18.9
1.46 (0)	1.9 (1.414)	72.6 ± 0.6	6.3 ± 0.2	361.9 ± 12.6	474.3 ± 18.1	575.5 ± 74.1	42.6 ± 0.9	28.5 ± 0.4	365.6 ± 40.7	185.2 ± 10.6
1.46 (0)	0.95 (0)	64.4 ± 0.3	5.3 ± 0.4	320.4 ± 4.2	417.0 ± 7.1	519.8 ± 39.3	37.1 ± 0.1	28.3 ± 0.0	328.6 ± 19.6	161.0 ± 9.4
1.46 (0)	0.95 (0)	65.1 ± 0.1	5.7 ± 0.2	316.5 ± 8.8	412.1 ± 12.6	499.0 ± 56.7	36.1 ± 1.1	27.8 ± 0.0	316.1 ± 17.4	138.2 ± 20.2
1.46 (0)	0.95 (0)	62.5 ± 0.4	5.7 ± 0.3	306.4 ± 6.8	398.6 ± 8.6	482.3 ± 40.3	35.8 ± 0.2	28.2 ± 0.1	308.1 ± 21.3	145.3 ± 13.6

(*) In brackets, the coded values of the two factors.

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In addition, the osmo-treated model food was significantly supplemented with phenolics, in particular with flavan-3-ols. The monomers of this phenolic group were found in concentrations between 1231±126 and 2020±246 mg/kg, and the dimers between 386±107 and 832±214 mg/kg. In comparison with the values reported for commonly consumed fresh and processed foods, then, the content of osmo-treated food was similar to that of proanthocyanidin-rich food products (see Table 6.5).

Table 6.5. Proanthocyanidin content (mg/kg) of different food commodities

Commodity	Monomer [mg/kg]	Dimer [mg/kg]	Reference
Chocolate products	797.9±248.6	590.5±140.7	Adamson et al., 1998
	3633.3±632.9	3066.7±514.0	Gu et al., 2004
Nuts			
Hazelnuts	98.3±15.7	125.1±38.4,	Gu et al., 2004
Pecans	172.2±25.5	421.3±54.2	
Pistachio	109±43.5	132.64±18.0	
Fruits			
Plums	108.8±29.1	385.4±107.2	Gu et al., 2004
Nectarines	106.4±46.9	119.3±82.8	Tomas-Barberan et al., 2001
Cranberries	72.6±15.1	259.3±61.2	Pascual-Teresa et al., 2000
Raspberries	39.1±27.4	86.4±83.6	
Strawberries	37.1±8.0	52.6±18.9	

The coefficients of the second order model show that linear and quadratic terms significantly determined the extent of ΔM^{w} (Table 6.5), while the interaction between NaCl and sucrose did not have a major influence. Similarly, ΔM^{SS} is mainly controlled by linear and quadratic effects whereas the interaction term is not significant. The gain in individual solutes presented some differences: although linear, quadratic and interaction terms are significant on ΔM^{NaCl}, only linear and quadratic effects of sucrose molality determine ΔM^{SUC} and ΔM^{TPH}. In addition, ΔM^{TPH} and $-\Delta M^{w}$ were more sensitive to variation in the sucrose molality than the NaCl molality of the osmotic solution.

The effect of NaCl and the sucrose concentration on mass transfer during OT with ternary aqueous solutions has been extensively investigated for several food commodities, and quite a widespread conclusion is that NaCl enhances water loss while sucrose seems to reduce NaCl gain (Sacchetti et al., 2001; Ade-Omowaye et al., 2002). However, to investigate the effect of the osmo-active agent on mass transfer, we have to take into account that the gradient of a_w (directly correlated to osmotic pressure) between the solid food and the osmotic solution drives water transport during OT.

Table 6.6. Regression coefficients and analysis of variance of the second order polynomial model for water loss, total solid, NaCl, sucrose and total phenolic gain and their corresponding mass fraction as well as TEAC in the osmo-treated food. Standard errors (SE). t- and P-values of the regression coefficients are included

Coefficient	ΔM^{W}	ΔM^{SS}	ΔM^{NaCl}	ΔM^{SUC}	ΔM^{TPH}_{HPLC}	x^{NaCl}	x^{SUC}	Monomers[a]	Dimers[a]	TEAC
b_0	-0.1409**	0.2204**	0.0349**	0.2209**	0.002**	0.0323**	0.2993**	1338.9**	485.84**	46.2**
Linear										
b_1	-0.1061**	-0.1384**	-0.0049**	-0.1467**	-0.0003**	-0.0057**	-0.1336**	-225.9**	-129.9**	-11.1**
b_2	-0.0135*	0.0215**	0.0235**	n.s.	n.s.	0.0217**	n.s.	n.s.	n.s.	n.s.
Quadratic										
b_{11}	0.0139**	-0.0433**	n.s.	-0.046**	n.s.	0.0008**	-0.040**	n.s.	80.8**	4.03**
b_{22}	0.0082*	n.s.	-0.0007*	n.s.	n.s.	-0.0009**	n.s.	n.s.	n.s.	n.s.
Interaction										
b_{12}	n.s.	n.s.	-0.0033**	n.s.	n.s.	-0.0037**	n.s.	n.s.	n.s.	n.s.
R^2	0.9930	0.9956	0.9993	0.9915	0.7252	0.9994	0.9913	0.7833	0.8708	0.9396
S.E.	0.0091	0.0097	0.0007	0.0142	0.0002	0.0006	0.013	134.8	58.8	3.00
F	226.8	364.0	2204.5	187.2	4.2227	2700.3	181.4	5.8	10.8	24.9
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0355	<0.0001	<0.0001	<0.015	<0.0021	0.0001

* Effect significant at P<0.05 confidence level
** Effect significant at P<0.01 confidence level
[a] – mass fraction expressed in (mg/kg).

For water/NaCl/sucrose solutions, the contribution of each individual solute to mass transfer should be established from the results obtained with solutions of different compositions but constant values of a_w. When the experimentation does not fulfill this condition, NaCl has the greatest dehydration effect because of the important differences in molecular weight between NaCl and sucrose: a small increase in the NaCl mass fraction significantly changes a_w and leads to major water loss. Thus, the experimental design considered osmotic solutions within quite a narrow range of a_w (from 0.887 to 0.976). From this point on, every response variable was calculated from its corresponding response surface but only for osmotic solutions (water/NaCl/sucrose/phenolics) with a constant a_w. Assuming that phenolics did not contribute to a_w in the osmotic solution (Rózek et al., 2007), we calculated several combinations of sucrose and NaCl concentrations that led to a 0.935 a_w within the range stated for each of them in the experimental design (sucrose: 0-2.9 molal, NaCl: 0-1.9 molal). Chirife et al (1984; 1985) found that the models applied (Norrish, Pitzer and Ross) predict the a_w of aqueous binary and ternary NaCl and sucrose solutions with

deviations that can be even less than the error of the typical instrumentation available for measuring it.

Figures 6.3 and 6.4 plot variable responses after 8 h of OT with osmotic solutions of different NaCl/sucrose compositions but with a constant a_w of 0.935. Variable responses are plotted against y_{OS}^{SUC} which was calculated as:

$$y_{OS}^{SUC} = \frac{n_{OS}^{SUC}}{n_{OS}^{SUC} + n_{OS}^{NaCl}} \qquad (6.6)$$

where n_{OS} is the moles of each osmo-active solute in the osmotic solution.

Figure 6.3 shows that water loss ($-\Delta M^{W}$) increased with y_{OS}^{SUC} so sucrose has a higher dewatering effect than NaCl when agar gel is used as the model food. As far as solute impregnation was concerned, the ΔM^{SS} and sucrose mass fraction in the osmo-treated food also increased with y_{OS}^{SUC} while the NaCl mass fraction decreased. The maximum contents of sucrose and NaCl were 0.43±0.04 and 0.080±0.002 kg/kg, respectively, which were obtained with y_{OS}^{SUC} values of 1 and 0.

These impregnation results can be explained by considering the ratio of sucrose to NaCl moles in the osmo-treated food, n_F^{NaCl}/n_F^{SUC}. Figure 6.3 shows the exponential decrease in this ratio versus y_{OS}^{SUC}, which indicates that sucrose limits NaCl gain, particularly when y_{OS}^{SUC} is below 0.5. When y_{OS}^{SUC} increases slightly to 0.25, the ratio of NaCl to sucrose moles in the osmo-treated food drastically decreases to values close to 2. For values of y_{OS}^{SUC} above 0.5, the ratio of NaCl to sucrose moles in the osmo-treated food is lower than 1 (Figure 6.3). When y_{OS}^{SUC} increases to 0.75, the ratio of NaCl to sucrose moles in the osmo-treated food reaches values close to 0.333. These results are similar to those reported during the OT of carrot with water/NaCl/sucrose solutions of 0.91 a_w (Behsnilian et al., 2006) in which case the ratio of NaCl to sucrose moles in the osmo-treated carrot was 0.29 for osmotic solutions with 0.76 y_{OS}^{SUC} and above 1 for y_{OS}^{SUC} <0.5. These results show that when y_{OS}^{SUC} >0.5, the penetration of sucrose moles predominates and results in a higher mass fraction of sucrose than of NaCl and an increase in total solute gain (ΔM^{SS}). Regarding flavan-3-ol monomers and dimers, their penetration into the model food is limited by sucrose to a different extent.

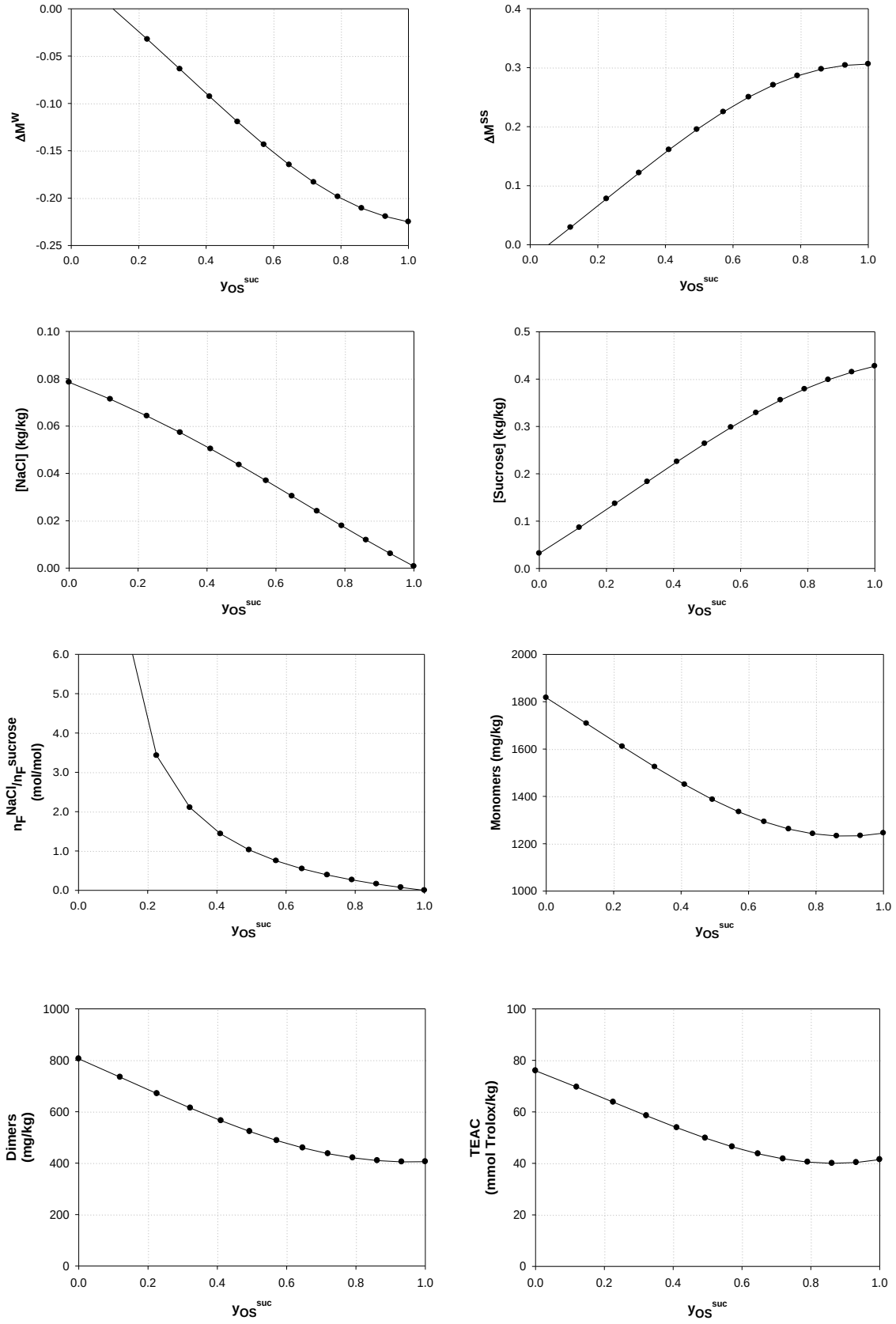

Figure 6.3. Mass changes of water (ΔM^{w}), gain in total solids (ΔM^{ss}) and NaCl, sucrose, flavan-3-ol monomer and dimer content, the ratio of sucrose to NaCl moles in the osmo-treated food, (n_F^{NaCl}/n_F^{SUC}), and TEAC versus y_{OS}^{SUC}. Data were obtained from the surface response for osmotic solutions of 0.935 a_w.

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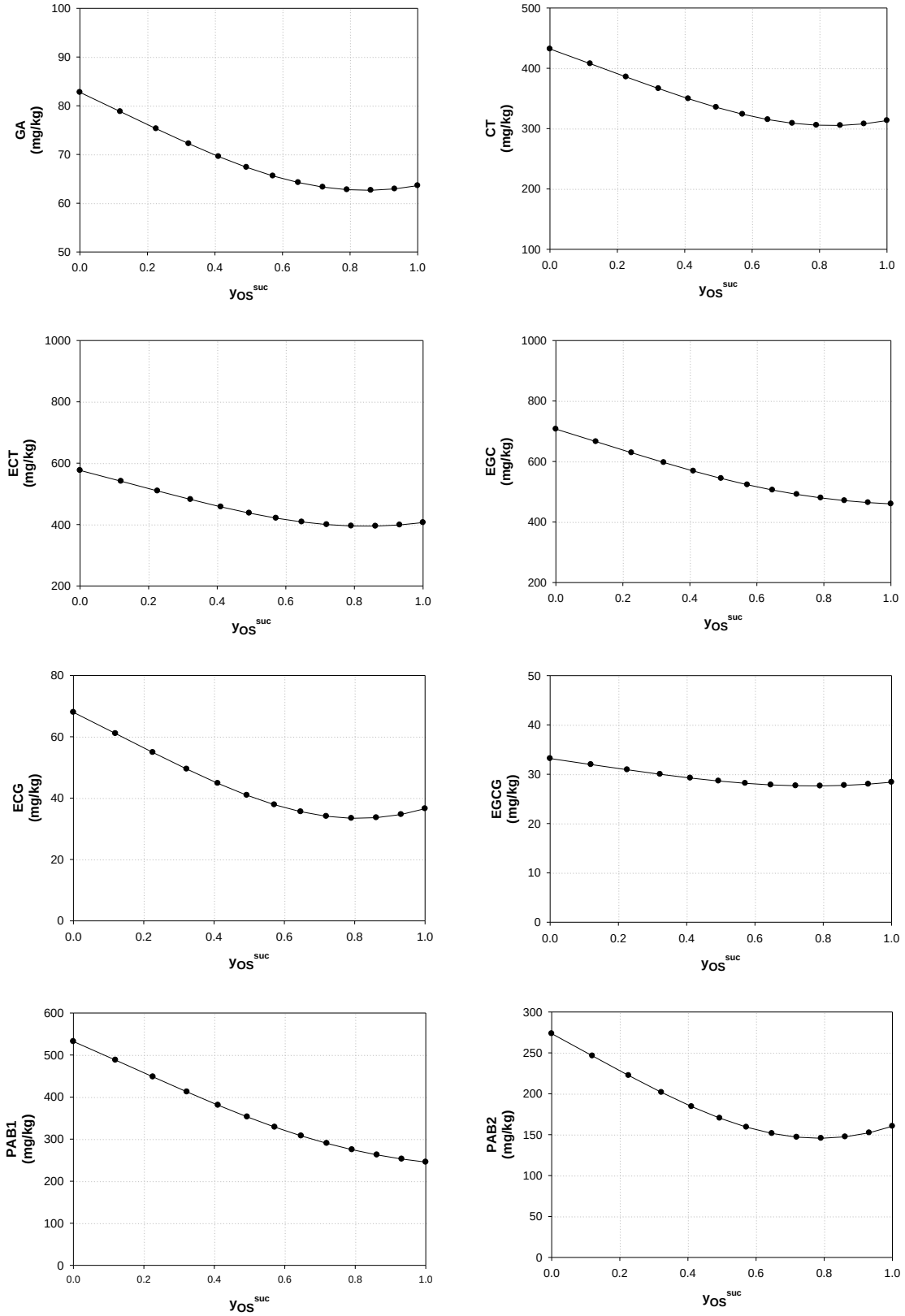

Figure 6.4. Content of GA, CT, ECT, EGC, ECG, EGCG, PAB1, and PAB2 versus y_{OS}^{SUC}. Data were obtained from the surface response for osmotic solutions of 0.935 a_w.

By increasing y_{OS}^{SUC}, monomer content decreases from 1816±461 to 1245±457 mg/kg while dimer content decreases from 806±200 to 406±199 mg/kg. When y_{OS}^{SUC} >0.75, monomer and dimer content reach minimum values of 1232±206 and 405±136 mg/kg, respectively.

Considering all this, a range of different osmo-treated model foods can be formulated with a very high content of flavan-3-ols (monomers and dimers), similar a_w but very different content of NaCl and sucrose. By using more than one osmo-active solute and adjusting the composition of the osmotic solution, OT can control not only the phenolics content but also the sensory properties of the end product. For instance, with y_{OS}^{SUC}=0.75 (3.7 % of NaCl and 41.3% sucrose in the osmotic solution), the osmo-treated food presents the lowest monomer and dimer impregnation, but a significant water loss ($-\Delta M^w$ = 0.19±0.01), relatively low NaCl (0.0220±0.0005 kg/kg) and high sucrose (0.37±0.01 kg/kg) content. Even though, sensory properties can be negatively affected by such a high gain in total solutes, NaCl impregnation may reduce the sensory effect caused by very high sucrose levels (Lerici et al., 1985).

6.3.2. Influence of the Osmo-active Agent on the Phenolic Profile of the Osmo-treated Food

The regression coefficients and analysis of variance of the fitted second order model for individual phenolics (Table 6.7) show that only linear and quadratic effects of sucrose molality have a significant influence on the mass fraction of flavan-3-ols quantified in the osmo-treated food.

In the case of hydroxybenzoic acids, a lack of fit was detected with PA content while in the GA mass fraction only the linear term of sucrose molality had an significant effect. The results obtained for the PA surface response are not considered in the following discussion of results. For each response variable we calculated the confidence limits (95%) of the predicted value. In case of individual phenolics, we found that the mean relative error of these estimations was about 15%, what was related to R^2 values between 0.62 and 0.91. As expected, the error distribution throughout the surface response was much higher at its lower and upper limits than around the central point. In spite of it, the surface response showed a clear behavior versus the composition of the osmotic solution which is thoroughly discussed next. From this point on, to improve clarity, error bars are not plotted in the graphs.The effect of the osmo-active solute on the impregnation of each individual phenolic was analyzed from their response surfaces.

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Table 6.7. Regression coefficients and analysis of variance of the second order polynomial model for content of individual phenolics determined by HPLC in the osmo-treated food (mg/kg). Standard errors (SE). t- and P-values of the regression coefficients are included

Coefficient	GA	PA	CT	ECT	EGC	ECG	EGCG	PAB1	PAB2	TPH_{HPLC}
b_0	65.66**	6.22**	324.69**	421.92**	526.25**	37.87**	28.21**	327.47**	158.4**	1896.67**
Linear										
b_1	-6.92*	n.s.	-46.44**	-65.34**	-100.99**	-11.25**	-1.95**	-94.21**	-35.67**	-363.23**
b_2	n.s. ***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Quadratic										
b_{11}	n.s.	n.s.	n.s.	33.02*	58.88*	6.20**	1.41**	50.38**	30.4**	205.8*
b_{22}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction										
b_{12}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s	n.s.	n.s.	n.s.
R^2	0.6215	0.4441	0.7478	0.7592	0.7961	0.9102	0.8870	0.8869	0.8256	0.8141
S.E.	6.13	0.79	29.69	41.06	59.96	3.40	0.853	38.22	21.33	198.43
F	2.63	1.28	4.74	5.04	6.25	16.22	12.57	12.55	7.57	7.01
P	0.1081	0.36	0.0261	0.022	0.0119	0.0005	0.0013	0.0013	0.0067	0.0085

***** Effect significant at P<0.05 confidence level.
****** Effect significant at P<0.01 confidence level
******* n.s. – not significant.

Only those osmotic solutions with a constant value of 0.935 a_w were considered (Figure 6.4). Although the trend is similar for all phenolics, the extent of the decrease in content versus y_{OS}^{SUC} depends on the kind of phenolic. The content of all individual phenolics, except EGC and PAB1, is minimum for y_{OS}^{SUC} values between 0.80 and 0.85. EGC and PAB1 contents decrease throughout the y_{OS}^{SUC} range and are minimum for osmotic solutes that contain only sucrose as the osmo-active agent.

Although sucrose reduced the phenolic infusion in all cases, the extent of the reduction was determined by the phenolic molecular weight. The penetration ratio of each phenolic, PR^{phj}, was calculated as:

$$PR^{phj} = \frac{x^{phj})_{\max} - x^{phj})_{\min}}{x^{phj})_{\max}} \qquad (6.7)$$

where x^{phj} is the mass fraction of an individual phenolic in the osmo-treated gel and the sub-indexes *max* and *min* indicate maximum and minimum values, respectively. Table 6.8 shows the maximum and minimum contents of individual phenolics and it can be seen that PR^{phj} increased with the phenolic molecular weight. For instance, the *PR* of GA (170.12 g/mol) was 24%, while for the flavan-3-ol dimers PAB1 and PAB2 (578.5

g/mol) it was 54 and 47%, respectively. Only EGCG had a *PR* that was low (17%) for its molecular weight (458.37 g/mol).

From the point of view of phenolic mass transfer, it is interesting to evaluate if 8 h of OT leads to equilibrium. Mass transfer is usually assumed to occur between the food liquid phase (i.e. food containing water and soluble components) and the osmotic solution. On this basis, the equilibrium criterion considered is that the food liquid phase and the osmotic solution are compositionally equal (Barat et al., 1998).

Table 6.8. Maximum and minimum mass fractions of individual phenolics, their penetration ratio, and *z* (content of each phenolic in the osmo-treated food expressed as mg/ kg food liquid phase) obtained with two osmotic solutions: y_{OS}^{SUC} =1 (only sucrose as osmo-active agent) and y_{OS}^{SUC} =0 (only NaCl as osmo-active solute). Data obtained from the surface responses for osmotic solutions of 0.935 a_w

					z [mg/kg fluid food phase]	
Phenolics	Molecular weight [g/mol]	$phj)_{max}$ [mg/kg]	$phj)_{min}$ [mg/kg]	PR^{phj} (%)	y_{OS}^{SUC} =0	y_{OS}^{SUC} =1
GA	170.12	83±21	63±7	24.3	86.2	66.1
CT	290.27	432±102	306±32	29.3	449.8	325.4
ECT	290.27	577±140	395±45	31.5	600.2	422.2
EGC	306.27	707±205	480±65	34.9	736.3	477.7
ECG	442.37	68±14	33±4	50.8	70.8	38.0
EGCG	458.37	33±3	28±1	16.7	34.6	29.5
PAB1	578.50	532±130	275±41	53.9	554.1	254.9
PAB2	578.80	274±73	146±23	46.8	284.6	166.5

Table 6.8 shows values of *z* (that is, the content of each individual phenolic in the osmo-treated food) expressed as mg/kg food liquid phase and obtained with two osmotic solutions: y_{OS}^{SUC} =1 (only sucrose as the osmo-active agent) and y_{OS}^{SUC} =0 (only NaCl as the osmo-active solute). In experiments performed with y_{OS}^{SUC} =1, the *z* values for all the phenolics are lower than for those in the osmotic solution, and the ratio of the content in the osmo-treated gel to that in the osmotic solution is above 50 % for all phenolics except for EGC and PAB1. When y_{OS}^{SUC} =0, *z* values were higher and the ratio of the content in the osmo-treated gel to that in the osmotic solution was 78% on average. For ECG, *z* values were higher than their content in the osmotic solution. Besides the errors of the predicted values calculated from the surface response, this result can also be explained if we consider that GSE (the actual source of the phenolics used in the osmotic solution) is a complex mixture of flavan-3-ols with different degrees of polymerization. Such a high content of ECG in the food liquid phase may be because the polyphenolics with a high number of flavan-3-ol units hydrolyzed (Wollgast et al.,

2000). The low pH (3.4) of the osmotic solution together with agitation during OT may promote this reaction pathway. Taking all this into account, we can conclude that the rate of phenolic mass transfer was higher when NaCl and not sucrose was the osmo-active solute. In addition, when sucrose is present in the osmotic solution, it seems to hinder the penetration of flavan-3-ols, in particular, of those with higher molecular weights (e.g. PAB1 and PAB2).

6.3.3. Antioxidant Properties and Their Correlation with the Phenolic Profile

The antioxidant properties of the osmo-treated food (in particular, its anti-radical scavenging capacity) were determined *in vitro* by TEAC. Although the correlation between TEAC and the real antioxidant capacity *in vivo* is still controversial, the considerable amount of TEAC data now available on many fruits and vegetables means that TEAC is now extensively used to compare the antioxidant scavenging capacities of many different food commodities.

The analysis of the regression coefficients of the second order model showed that only the linear and quadratic coefficients of the sucrose molality are significant, as was observed with the individual phenolics analyzed (Table 6.5). These results indicate that sucrose is the osmo-active solute that mainly limits phenolic penetration and, then, the antioxidant scavenging capacity of the osmo-treated gel.

As with the individual phenolics, to discuss the effect of the osmo-active solute on TEAC, we took into consideration the results obtained from the TEAC response surface for osmotic solutions with a constant a_w of 0.935. Figure 6.3 shows that TEAC values decreased with y_{OS}^{SUC} from 76.0±10.2 to 40.0±4.6 mmol Trolox/kg. Comparing these results with average TEAC values for some fruits, we can establish that the TEAC of the osmo-treated gel was higher than that of fruits with a very high free radical scavenging activity. Blackberry and raspberry, for instance, have reported TEAC values of 20.24 and 16.79 mmol Trolox/kg of FW, respectively (Pellegrini et al., 2003). Previous results (17) obtained with an osmotic solution of concentrated grape juice with a mass fraction of soluble solids of 50% (0.935 a_w) showed TEAC values of 66.3 mmol Trolox/kg in an osmo-treated gel. However, the total phenolic content of the concentrated grape juice was 13152±276 mg GAE/kg, which is twice that of the total phenolic content in the osmotic solution (6300±45 mg GAE/kg) used here.

To determine the extent to which the individual phenolics identified describe TEAC in the osmo-treated food, correlations between TEAC and the phenolic profile were

determined. Linear regression analysis (Table 6.8) showed that the total phenolics identified by HPLC have a significant effect on TEAC (R^2 = 0.6182, P<0.001). In particular, flavan-3-ol dimers (R^2 = 0.6618, P<0.001) seem to make a slightly higher contribution to the radical scavenging capacity than the flavan-3-ol monomers (R^2 = 0.5844, P<0.005) while the hydroxybenzoic acid, GA, made a significant but lower contribution (R^2 = 0.4672, P=0.007). All flavan-3-ol monomers made a significant contribution, particularly ECG followed by EGCG, CT, ECT, and EGC. Of the flavan-3-ol dimers both PAB1 and PAB2 had very similar correlations with TEAC. Similarly, in a study of the correlations between TEAC and phenolic content of GSEs obtained from several grape varieties (28), flavan-3-ol dimers made a higher contribution than monomers.

Table 6.9. Linear regression analysis of antioxidant capacity, TEAC, versus individual and total phenolic content

Phenolics	**a***	**b***	**R^2**	**F**	**P****
GA	0.839	-7.32	0.4672	10.52	0.0070
CT	0.157	-3.97	0.5752	16.25	0.0017
ECT	0.111	0.19	0.5728	16.09	0.0017
EGC	0.069	10.52	0.5647	15.57	0.0019
ECG	0.786	16.40	0.7379	33.79	0.0001
EGCG	3.836	-61.97	0.6346	20.84	0.0006
PAB1	0.089	17.82	0.6880	26.46	0.0002
PAB2	0.182	17.41	0.5759	16.29	0.0016
Total phenolics$_{HPLC}$[a]	0.021	7.17	0.6182	19.42	0.0009
Flavan-3-ol Monomers	0.032	3.65	0.5844	16.87	0.0015
Flavan-3-ol Dimers	0.061	17.16	0.6618	23.48	0.0004

[a.] Total phenolics determined using HPLC
* **a** (mmol Trolox/mg of phenol) and **b** (mmol Trolox/kg) are the slope and the intercept.
** P-values of the regression coefficients.

The extent of phenolic infusion significantly increased the antiradical scavenging capacity of the osmo-treated gel, while all the flavan-3-ols detected and the hydroxybenzoic acid, GA, also made significant contributions.

Further applications of OT to infuse solid foodstuffs with grape phenolics should take into consideration other sources of grape phenolics so that the extent of infusion in different operating conditions can be determined. In addition, the results obtained with GSE and grape juice as a source of phenolics should be validated when real structured foods are used. The stability of phenolics during further processing steps and the sensory properties of the end product require further investigation

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CHAPTER 7

*EFFECT OF AIR DRYING ON THE STABILITY OF GRAPE PHENOLICS INFUSED INTO FRUITS AND VEGETABLES BY OSMOTIC TREATMENT**

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7.1. INTRODUCTION

At present, there is increasing interest in exploitating grape by-products from winemaking to obtain potentially bio-active phenolic compounds (Louli et al., 2004; González-Paramás et al., 2004). In recent years, the use of rich-in-phenolic grape seed extracts as a nutritional supplement with antioxidant properties has started to become popular. With regard to their pharmacological properties, these phenolics have been shown to be active, in *in vitro* studies, against oxidation of the low-density lipoproteins, at the same time as they appear to demonstrate antiulcer, anticarcinogenic, antimutagenic, and antivirial activity (Meyer et al., 1997; Plumb et al., 1998; Teissedre et al., 1996; Saito et al., 1998).

Osmotic treatment (OT), also known as osmotic dehydration or dewatering-impregnation soaking, is a unit operation that involves immersing a solid food in a hypertonic aqueous solution leading to the loss of water and a solute transfer from the solution into the food. OT has been reported as a feasible treatment for incorporating physiologically active compounds into plant tissues without destroying the initial food matrix (Alzamora et al., 2005).

Using OT, the homogenous structure of a model food made of agar gel was supplemented with grape phenolics when a concentrated red grape must was used as the osmotic solution (Rózek et al., 2007). Furthermore, a range of different osmo-treated model foods were formulated with a very high content of flavan-3-ols (monomers and dimers), similar water activity (a_w) but very different contents of NaCl and sucrose by using a commercial grape seed extract as a source of phenolics and more than one osmo-active solute in the osmotic solution (sucrose and sodium chloride) (Rózek et al., 2008).

Typically OT is applied to biological materials of plant origin, such as fruits and vegetables that consist of tissues or organizations of cells with different characteristics and complexity. Water and solute mass transfer strongly depends not only on the properties of the osmotic solution (including type and concentration of osmo-active solute, osmotic gradient) and working pressure, but also on the structure of the solid food to be treated (Spiess et al., 1998). Bearing this in mind, using OT to formulate any type of solid food supplemented with grape phenolics requires investigating how plant tissue structure affects mass transfer of grape phenolics. With this objective, we have

investigated three plant commodities with significant differences in their structure: apple, banana and potato.

Overall, raw plant tissue is a very heterogeneous material structurally and can be pictured as a solid matrix in which liquid enclosed in semi-permeable membranes is immobilized. In apple, the parenchyma cells and air spaces are arranged in a different way in outer and inner apple tissue (Lewicki et al., 2000) and show differences in cell and intercellular dimensions between the inner (close to the core) and outer (close to the skin) parenchyma (Vincent, 1989; Khan et al., 1993). Similarly, banana tissue is basically composed of parenchyma with irregularly shaped cells with well-defined thin cell walls, interspersed with air spaces. Unlike apple, most of the cells contain starch grains, with the size and number of these grains varying from cell to cell. The cells differ in size, becoming smaller where they form vascular bundles. Most of the cells forming vascular bundles do not contain any starch grains. The vascular bundle cells are linked together to form a wall that surrounds a duct, most probably a xylem vessel (Kyamuhangire et al., 2006). Potato tuber is composed of the internal parenchyma and potato pith. Although the parenchyma cells which contain starch grains represent the major part of the tuber, the pith with medullary rays of cells with different characteristics have a significant role on the structural properties of potato tissue. The internal parenchyma cells are bigger than potato pith cell which contain relatively few starch grains (Sadowska et al., 2008).

As has been mentioned, there is increasing interest in using OT to produce intermediate moisture products of improved quality rather than using it as a preserving method. Consequently, the osmotic process has received considerable attention as a pre-treatment to further processing (Karathanos et al., 1995; Spiess et al., 1998). The combination of air drying with OT has been widely reported because of the improvements in the quality of the end product and energy savings. The sugar intake obtained in some fruits by OT reduced or even avoided sulphating and stabilized plant pigments and flavor during subsequent air drying and storage (Torreggiani, 1995).

In contrast, little is known about the stability of polyphenols during air drying. The effect of drying temperature has been studied in red grape pomace peels: drying at 60 ºC did not significantly affect either the total phenolic content or the antioxidant activity, whereas temperatures above 100 ºC significantly reduced both of these (Larrauri et al., 1997). In the case of strawberries, air drying at temperatures of 60 ºC for 220 min leading to a final moisture content of 0.05 kg/kg dry product reduced the total phenolic

content by an average of 28% while the Trolox Equivalent Antioxidant Capacity reduced by 58% (Böhm et al., 2006). Anthocyanin and flavonol content decreased significantly when prunes were air-dried at 85 ºC until they had a final moisture content of 0.25 kg/kg dry product, although their antiradical scavenging capacity increased (Piga et al., 2003).

The main objective of this study was to apply OT to infuse phenolic compounds into plant tissue and to evaluate their stability after a post-treatment such as convective air drying. To do this, a model food made of agar gel and three plant commodities with significant differences in their tissue structure (two fruits, apple and banana, and one vegetable, potato) were osmo-treated and subsequently air-dried. In the osmotic solution, sodium chloride and sucrose were used as single osmo-active solutes when treating fruits and vegetables, respectively, while a commercial grape seed extract was the source of phenolics.

7.2. MATERIALS AND METHODS

7.2.1. Fruit, vegetable and model food procedures

Fresh apples (*Malus pumila*, var. Granny Smith), bananas (*Musa acuminata*, var. Cavendish) and potatos (*Solanum tuberosum*, var. Monalisa) were purchased from a local market. These varieties were chosen because they are readily available throughout the entire year at a fairly constant quality. The same lot of fruits was used in each experiment in order to minimize biological variability due to the age and cellular structure. Apple, banana and potato were washed and hand peeled. The tips from each end of the banana were discarded and the apple core was taken out.

As a model food, an agar-agar gel was prepared with 4% (w/w) agar-agar (Scharlau, Spain), 9.6% (w/w) sucrose, and distilled water. The mixture was heated to 95 °C in a microwave oven until the agar-agar was completely dissolved. Gelation was achieved by cooling at room temperature. The gel was then stored at 6 ± 2 °C and used within 2 days. Apple, banana, potato and model food samples were cut in 1 cm side cubes.

7.2.2. Osmotic solutions

A commercial grape seed extract (Vitisol® supplied by Berkem, Gardonne, France) was used as a source of phenolic compounds. In all experiments, the mass fraction of the total phenolics in the osmotic solution was set to 6300 ± 45 mg GAE/kg. Solutions with 50 % (w/w) sucrose (refined, 99.9% sucrose) were used during the OT of apple and banana, while solutions with 10 % (w/w) sodium chloride (J.T. Baker, Germany) were used to treat potato. At these concentrations, all osmotic solutions presented a similar water activity of 0.935 ± 0.010.

7.2.3. Osmotic treatment

The experimental set-up consisted of two parts: a basket to allocate the samples and a vessel to be filled with osmotic solution. The basket consisted of five shelves on which the fruits and vegetable samples were placed without being in contact with each other. Contact with the osmotic solution was guaranteed by the height of the shelves and the cavities in all the walls of the basket and the shelves. The sample cubes were placed in a basket and then immersed into the osmotic solution vessel. Then the vessel was placed in a magnetic stirrer. The agitation level was chosen in order to make the surface mass transfer negligible.

About 40 g of apple and banana and 50 g of potato and model food were weighed and placed in the OT basket. The basket was then submerged in 1 L of osmotic solution. The solution/food ratio (w/w) was always higher than 20:1, preventing significant alteration of the solution concentration during the OT. The samples were processed for 0.5, 1, 2, 4 and 8 hours. The temperature was maintained at 25 ± 2 °C. All experiments were run under atmospheric pressure. Each experiment was performed in duplicate. After OT, sample cubes were removed, drained and flushed with deionized water to eliminate the sugar and/or salt that had accumulated on their surface. They were then blotted with absorbing paper and weighed. After this, the moisture content and a_w of the osmo-treated samples were determined. The content of individual phenolic compounds impregnated into the model food was determined in samples after 8h of OT.

7.2.4. Air drying

The osmotically pre-treated apple, banana, potato and agar gel samples were placed in a laboratory scale drier. This basically consisted of a through flow chamber with controlled temperature and airflow velocity (Mulet et al., 2000). Experiments were conducted at 55 °C with an air rate of 4 m/s, which ensured that mass transfer was controlled by the internal resistance. The initial load of osmo-treated samples to be dried was approximately 100 g. In order to characterize drying kinetics, the weight of the samples was monitored during drying, and the final moisture content was determined at least in triplicate. The samples were dried until a constant weight was reached. Each experiment was carried out in duplicate. Dried samples were stored at 5 °C for 1 week until the phenolic extraction process.

7.2.5. Analytical methods

7.2.5.1. Determination of moisture content and a_w

The moisture content of fresh, osmo-treated and osmo-air dried samples was determined gravimetrically following the official AOAC method 920.151 (1998). A hygrometer was used to measure the water activity of the osmotic solution, and the apple, banana, potato and agar gel (Novasina, IC-500, AW-LAB).

7.2.5.2. Determination of sucrose and sodium chloride

About 5 g of milled fresh and osmo-treated samples were dissolved in 100 mL of miliQ water, placed on magnetic stirrer and agitated for 2 hours. After filtration, the sucrose and sodium chloride content in the aqueous extract were determined. The total sucrose content was determined by the Rebelein method (Barceló, 1990) using a GAB kit for sugar analysis (GAB Sistemática Analítica S.L., Barcelona, Spain). The sodium chloride content was quantified according to Mohr's method (James, 1995).

7.2.5.3. Extraction of phenolic compounds

Phenolic compounds were sequentially extracted from fresh, osmo-treated and osmo-air-dried food in order to determine the total and individual phenolics. A ground sample (5 g) was extracted sequentially with a 30 mL solution of methanol/water (50:50, v/v)

and a 30 mL solution of acetone/water (50:50, v/v), for 1 h in each extraction solvent and at room temperature. Each extraction was carried out in duplicate.

7.2.5.4. Determination of total phenolic content

The total phenolic content (TPH) of the osmotic solution and osmo- and osmo-air-dried food extracts was determined spectrophotometrically (CECIL, CE2021) by Folin-Ciocalteu's colorimetric method (Singleton et al., 1965). TPH was expressed as the gallic acid equivalent using the standard curve prepared at different concentrations of gallic acid. Data presented are the average of two measurements for each extract.

7.2.5.5. Phenolic profile in osmo-treated model food – HPLC Analysis

Individual phenolics were identified and quantified using liquid chromatography HPLC (Hewlett-Packard, HP/Agilent, Wardborn, Germany) equipped with ChemStation software. A Supelcosil column LC-18 (25 cm x 4.6 mm) with a particle size of 5 µm was used. A mean injection volume of 100 µL was provided by an automatic injector, HP 1000. Prior to HPLC analysis, the gel extracts were taken to dryness by vacuum evaporation ($T \leq 40°C$), re-suspended in miliQ water and filtered through 0.45 µm syringe filters (Teknokroma, Barcelona, Spain). The extracts were analyzed according to the method previously reported by Rózek et al., (2008).

Gallic acid (GA), protocatechuic acid (PA), (+)-catechin (CT), (-)-epicatechin (ECT), (-)-epicatechin 3-*O*-gallate (ECG), (-)-epigallocatechin 3-*O*-gallate (EGCG), (-)-epigallocatechin (EGC), procyanidin B1 (PAB1) and procyanidin B2 (PAB2) were purchased from Sigma-Aldrich (Steinheim, Germany). Results were expressed as milligrams of phenol per kilogram on a wet basis. All determinations were performed in duplicate.

7.2.5.6. Trolox Equivalent Antioxidant Capacity Assay – TEAC

Antioxidant capacity of the prepared extracts was assessed according to the ABTS decolorization assay (Re et al., 1999), which is based on to what extent antioxidants inhibit the absorbance of the radical cation of 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS). This compound has a characteristic long wavelength absorption spectrum showing maxima at 734 nm. The results were expressed as mmol of Trolox equivalents per kilogram on a wet or dry basis.

7.2.6. Calculation procedure

7.2.6.1. Mass transfer parameters

The OT kinetics of apple, banana, potato and agar gel cubes were analyzed by calculating the following parameters: water loss ($-\Delta M^{w}$), soluble solid gain (ΔM^{ss}), gain in moles of osmo-active solute (ΔN^{ss}) and total phenolic gain (ΔM^{TPH}) according to the equations (7.1) to (7.4),

$$\Delta M^{w} = \frac{M_t \cdot x_t^{w} - M_0 \cdot x_0^{w}}{M_0} \qquad (7.1)$$

$$\Delta M^{ss} = \frac{M_t \cdot x_t^{ss} - M_0 \cdot x_0^{ss}}{M_0} \qquad (7.2)$$

$$\Delta N^{ss} = \frac{M_t \cdot n_t^{ss} - M_0 \cdot n_0^{ss}}{M_0} \qquad (7.3)$$

$$\Delta M^{TPH} = \frac{M_t \cdot x_t^{TPH} - M_0 \cdot x_0^{TPH}}{M_0} \qquad (7.4)$$

where M is the mass of food, x is the mass fraction and n is the molar fraction of each component in the food. The subscripts *0* and *t* indicate initial immersion time and immersion time at time *t*. The superscripts *w*, *ss*, and *TPH* indicate moisture, soluble solids and total phenolics. The mass fraction of each component is expressed as kg/kg on a wet basis.

7.2.6.2. Effective diffusion coefficients

Assuming that the samples behave like an isotropic structure and considering the external mass transfer negligible compared to the internal resistance, the solution of Fick's second law for cubical geometry was used to model the mass transfer of water and soluble solids during OT. The initial and boundary conditions assumed that the sample initial moisture and soluble solid content was uniform, that is, the solid food was symmetrical regarding the mass transfer direction, and the sample surface was in the thermodynamic equilibrium. Mass transfer was assumed to occur between the food liquid phase (i.e. food containing water and soluble components) and the osmotic solution. According to this, the equilibrium criterion was that the food liquid phase and the osmotic solution should be compositionally equal (Barat et al., 1998). The effective diffusion coefficients were calculated by means of the analytical solution of Fick's

second law applied to cubical configuration. The total amount of each diffusing substance crossing the cube surface during a period of time t is given by Crank, 1995.

$$Y_r^j = \frac{\left(z_t^j - z_\infty^j\right)}{\left(z^j - z_\infty^j\right)} = \left\{ \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 \frac{\pi^2 D_{ej}}{4a^2} t \right] \right\}^3 \qquad (7.5)$$

where Y_r^j indicates the ratio of each diffusing compound or group of compounds; z is the mass fraction in the food liquid phase; the subscripts 0,∞, and t represent initial concentrations, at equilibrium, and at any time; D_e is the effective diffusion coefficient, and a is half the thickness of the cube.

Moisture diffusion coefficients (D_{ew}^{AD}) during convective drying were calculated by applying the Crank solution of Fick's second law to a cubical geometry (see equation 7.5). The same assumptions were made as for water mass transport during OT, but the moisture ratio was calculated according to the following equation (Simal et al., 1997):

$$\Psi(t) = \frac{W - W_e}{W_c - W_e} \quad (7.6)$$

where W is a moisture concentration in the solid, W_e is the moisture equilibrium (in the conditions applied, it is the moisture content at the end of the air drying process) and W_c is the critical moisture content and corresponds to the moisture content of the solid at the beginning of the air drying process (in this case the moisture content after OT).

The values of D_e and D_{ew}^{AD} were determined by regressing experimental data to equation 4. The fitting was performed with SigmaPlot version 10.0 by applying the Marquardt-Levenberg algorithm. The regression coefficient, R^2, was used to measure how well the regression model described the data.

7.2.6.3. Determination of Peleg constants

The model proposed by Peleg (1988) and redefined by Palou et al. (1994) was employed to fit the progress of total and individual phenolic content during OT. Peleg's equation is an empirical model with two parameters initially established to describe sorption isotherms that approach equilibrium asymptotically:

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$$\frac{t}{x_t^j - x_0^j} = k_1^j + k_2^j t \quad (7.6)$$

where *x* is the mass fraction of each component expressed as kg/kg on a wet basis. Sub-indexes *0* and *t* indicate initial conditions and conditions at time t of treatment and super-index *j* indicates any of the components transferred. The constants k_1 and k_2 are the Peleg rate constant and Peleg capacity constant, respectively and are determined by fitting experimental data to equation 7.6. The Peleg rate constant, k_1, relates to the initial rate of mass change of any component:

$$\left.\frac{dx_t^j}{dt}\right|_{t=0} = \pm\frac{1}{k_1^j} \quad (7.7)$$

The Peleg capacity constant k_2 relates to the contents at equilibrium, that is, the maximum phenolic content attainable after OT. As time $t \to \infty$, the equation (7.2) gives the relation between equilibrium (∞) conditions and k_2.

$$x_\infty^j = x_0^j \pm \frac{1}{k_2^j} \quad (7.8)$$

7.3. RESULTS AND DISCUSSION

7.3.1. Fresh food and grape seed extract

Table 7.1 shows the moisture and total phenolic content, the water activity and the TEAC of fresh apple, banana and potato. The highest concentration of total phenolics was found for apple, 1257 ± 5.67 mg GAE/kg FW, followed by banana and potato, 1047 ± 34.8 and 939.6 ± 37.3 mg GAE/kg FW, respectively.

Table 7.1. Moisture and total phenolic content, a_w and TEAC of fresh foods

	Moisture content (%, w/w)	Water activity (a_w)	Total phenolics content (mg GAE/kg)	TEAC (mmol Trolox/kg)
Apple	86.8±0.47	0.982	1257±5.67	9.39 ± 1.00
Banana	74.2±0.58	0.978	1047±34.8	5.58 ± 0.03
Potato	84.9±0.98	0.984	939.6±37.3	4.98 ± 0.40

The total antioxidant capacity was also determined and the trend was similar to that of total phenolics content. The TEAC values for apple, banana and potato were 9.39 ± 1.0 mmol Trolox/kg FW, 5.58 ± 0.03 and 4.98 ± 0.4 mmol Trolox/kg FW, respectively. These results are close to the total phenolic content reported for apple (1183 ± 14.0 mg GAE/kg FW) and banana (1128 ± 67.0 mg GAE/kg FW) while higher than the content found in potato (352.8 ± 5.6 mg GAE/kg FW) (Chun et al., 2005).

Commercial grape seed extract was chosen as a source of phenolic compounds in the osmotic solution. The total polyphenol content was set to obtain a phenolic mass fraction of 0.63%. The content of individual hydroxybenzoic acids, flavan-3-ol monomers and dimers content were determined by HPLC (Figure 7.1). Flavan-3-ol monomers were the major group quantified followed by flavan-3-ol dimers and hydroxybenzoic acids, which represented 69%, 28% and 3%, respectively, of all individual phenolics quantified by HPLC.

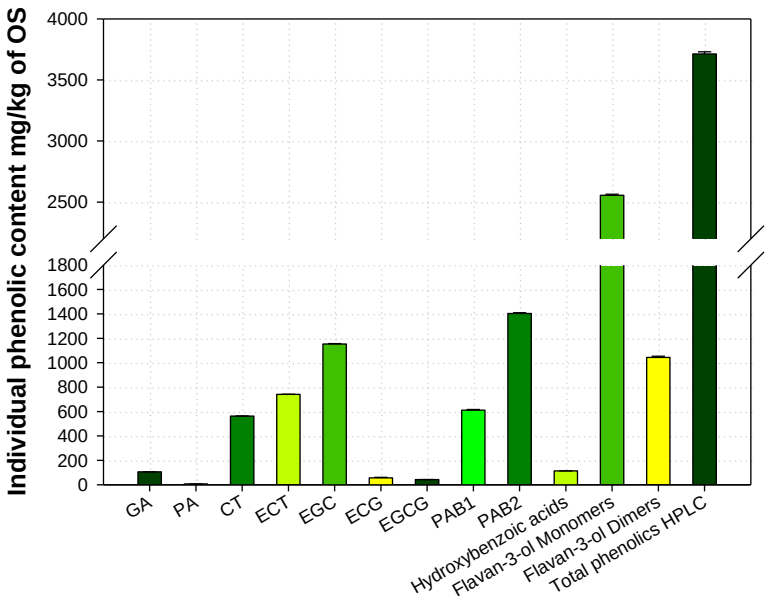

Figure 7.1. Phenolic profile of grape seed extract.

Moreover, the individual phenolics of low molecular weight were almost 59% (w/w) of the total phenolics determined by Folin-Ciocalteu's method.

7.3.2. Influence of food structure on grape phenolic infusion and mass transfer during OT

Figure 7.2 shows the influence of food tissue and type of osmo-active solute on overall mass increments during OT. Loss of water, $-\Delta M^{W}$, soluble solid gain, ΔM^{SS}, mole solute gain, ΔN^{SS}, and total phenolic gain, ΔM^{TPH}, during OT of apple, banana, potato and the model food increased with the immersion time. Food structure strongly determined mass transfer of water, soluble solutes and grape phenolics.

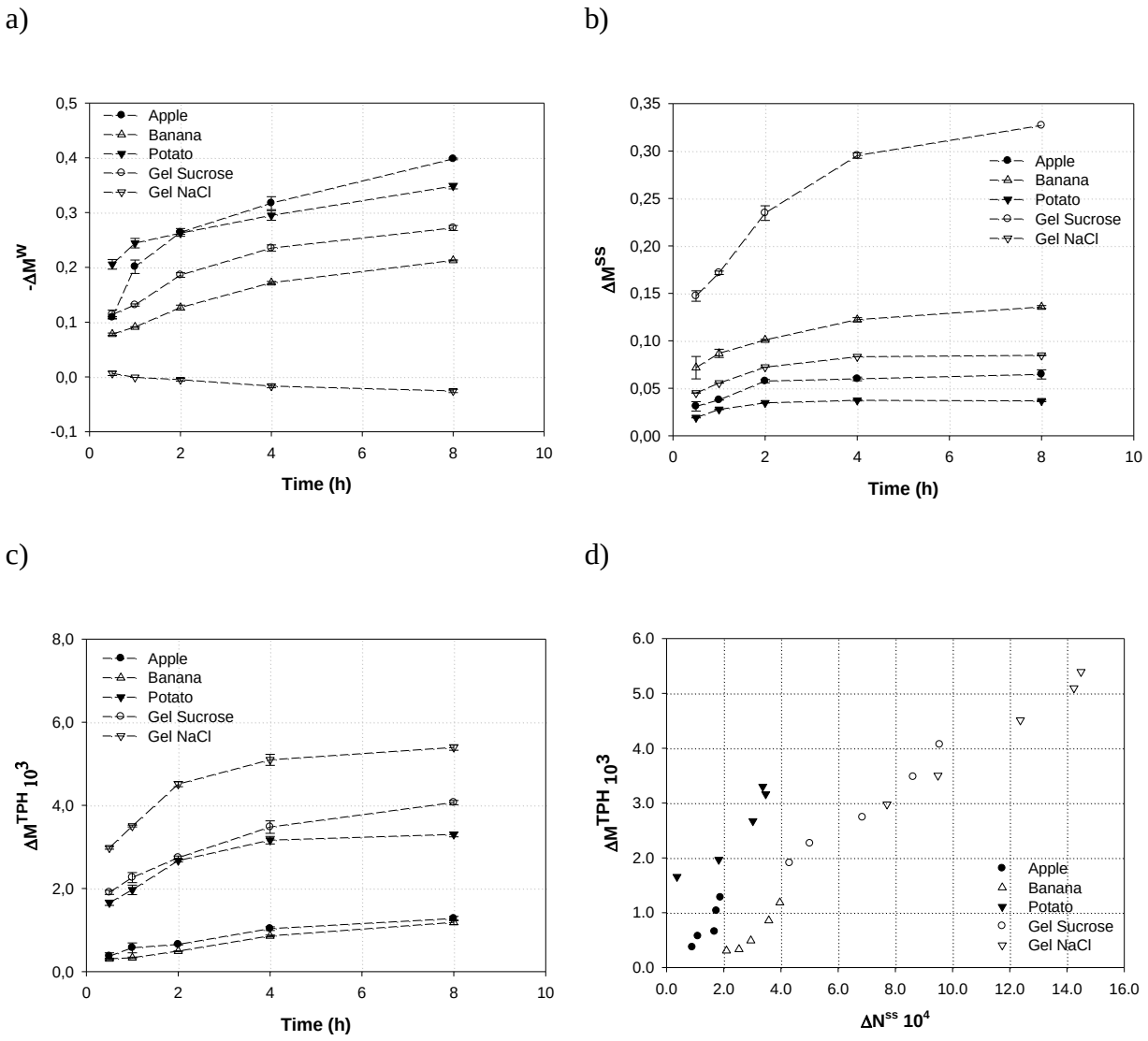

Figure 7.2. Mass changes during OT: a) water loss ($-\Delta M^{W}$), b) gain of osmo-active solute (ΔM^{SS}), c) gain in total phenolics, and d) total phenolics versus the gain of osmo-active solute in moles (mean and standard deviation of experiments performed in duplicate).

When using sucrose as an osmo-active solute, apple tissue showed the highest water loss (0.40 ± 0.001) and the lowest sucrose (0.065 ± 0.005) and total phenolic gains (0.0012 ± 0.00005) compared to those of the model food and banana tissue. In terms of phenolic gain, both apple and banana showed similar values, 0.13% and 0.12% for apple and banana, respectively, after 8 h of OT. As expected, the resistance to sucrose and grape phenolic mass transfer of both apple and banana tissues was higher than that of the model food, which showed a much more extensive sucrose and total phenolic gain. Similarly, OT with sodium chloride of potato led to lower sodium chloride and total phenolic gains than those observed in the model food.

Previous results with a similar model food (agar gel) revealed that phenolic infusion was coupled to osmo-active solute impregnation (Rózek et al., 2008). Figure 7.2d shows that, in the model food, the mole sodium chloride gain was much higher than that of sucrose and, simultaneously, ΔM^{TPH} increased almost linearly with the mole solute gain. On the other hand, apple and banana showed that a similar gain of total phenolics corresponded with a sucrose gain in banana that was twice that observed in apple tissue (Figure 7.2d). Moreover, ΔM^{TPH} obtained with sodium chloride in potato was between 2.5 and 7 times higher than that obtained with sucrose in apple and banana.

Several structural parameters may explain the higher solute infusion observed in the model food compared with that of plant tissues, with the cell membrane having a key role as a selective barrier. In addition, the cell membrane has been shown to be permeable not only to water but also to some solutes to an unlikely extent, which may justify the differences in solute infusion observed among the three plant tissues investigated depending on the osmo-active solute used. For instance, the reflection coefficient (the relative permeability of a particular membrane to a particular solute) of sodium chloride (σ=0.5-0.6) is lower than that of sucrose (σ=1) which cannot cross the cell membrane (Behsnilian et al., 2006). Potato showed the highest ΔM^{TPH} to ΔN^{ss} ratio while the mole sodium chloride gain was similar to the mole sucrose gain observed in apple and banana. These results may be attributed to the high phenolic permeability of potato tissue.

However, other structural parameters rather than cell membrane permeability may control solute mass transfer. In the case of apples, it was reported that processing time favors the release of the gas included in the tissue. The gas release decreases the internal

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resistance to the mass transport, and thus, enhances the dewatering effect and limits solute infusion (Behsnilian et al., 2006).

To describe and compare mass transfer kinetics during OT in different operating conditions, experimental data were fitted to the diffusional model (eq. 7.5) and Peleg's equation (7.6). The changes in moisture, soluble solids and total phenolics in apple, banana, and potato were calculated as dimensionless values related to the initial and equilibrium conditions in order to calculate the effective diffusion coefficients (Figure 7.3) Table 7.2 shows effective diffusion coefficients and Peleg constants of moisture, osmo-active solute and total phenolics.

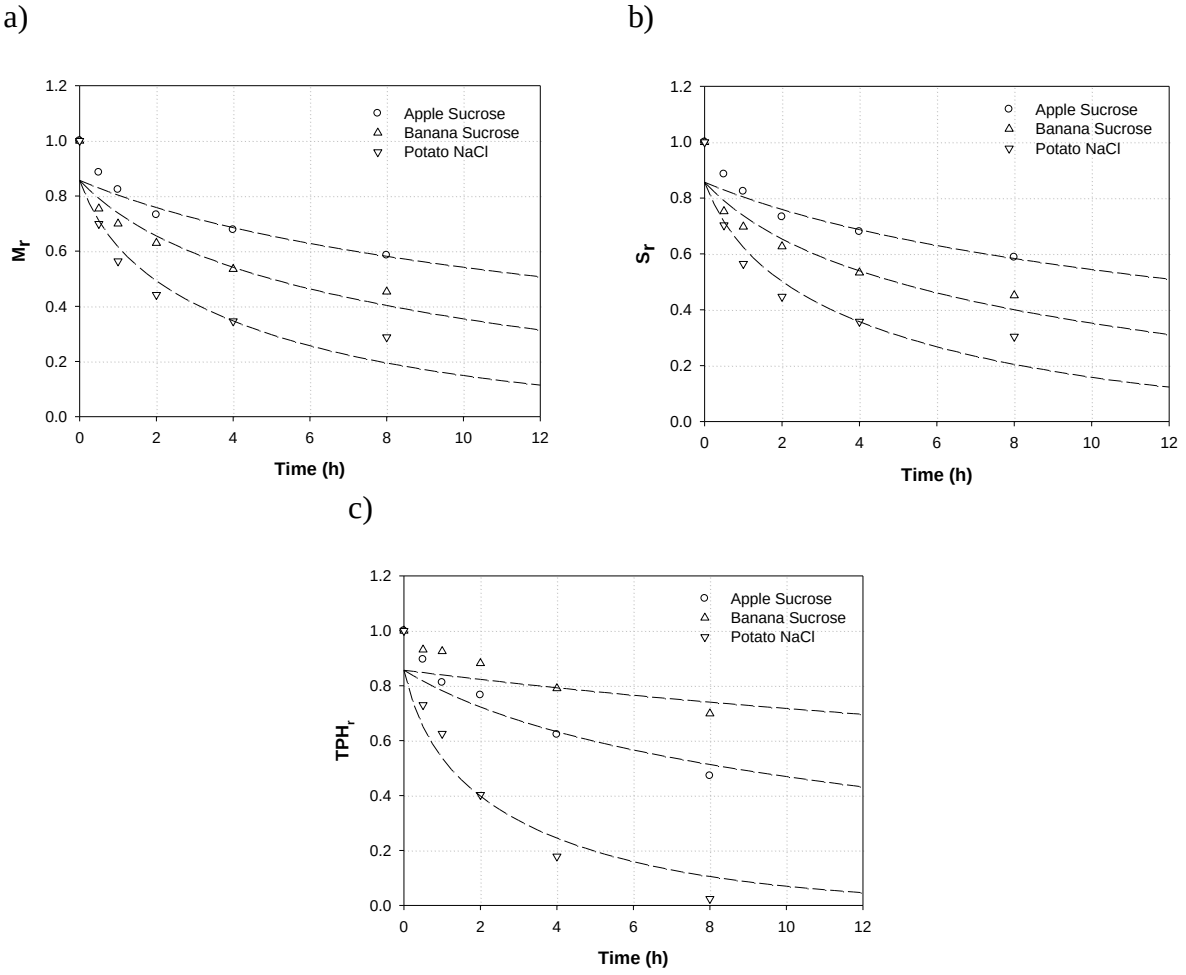

Figure 7.3. Ratio of moisture (a), soluble solid (b) and total phenolic content (c) during OT with sucrose and sodium chloride OSs.

According to the regression results (Table 7.2), the diffusional model did not describe appropriately mass transfer of total phenolics in banana while the progress of moisture,

osmo-active solute and total phenolic content during OT fitted well Peleg's equation in apple, banana and potato.

Table 7.2. Effective diffusion coefficients and their standard error of moisture, osmo-active solute and total phenolics during OT of apple, banana and potato

	$D_e\ 10^{10}$ (m^2/s)					
	Water	R^2	Osmo-active solute	R^2	TPH	R^2
Apple	1.87 ± 0.56	0.777	1.84 ± 0.56	0.774	2.71 ± 0.78	0.826
Banana	4.66 ± 1.01	0.854	4.72 ± 1.03	0.854	n.s.*	n.s.
Potato	1.91 ± 0.25	0.899	1.15 ± 0.24	0.888	1.91 ± 0.43	0.931
	Peleg rate ($1/k_1$, s^{-1})					
	Water		Osmo-active solute		TPH	
	$1/k_1\ 10^5$ (s^{-1})	R^2	$1/k_1\ 10^5$ (s^{-1})	R^2	$1/k_1 10^7$ (s^{-1})	R^2
Apple	2.56 ± 0.30	0.984	4.46 ± 0.78	0.994	3.02 ± 0.39	0.960
Banana	1.34 ± 0.34	0.880	5.86 ± 1.19	0.990	1.73 ± 0.34	0.890
Potato	3.85 ± 0.80		2.73 ± 0.04	1.000	7.38 ± 0.59	0.974
	Peleg capacity ($1/k_2$, kg/kg)					
	Water		Osmo-active solute		TPH	
	$1/k_2$ (kg/kg)	R^2	$1/k_2$ (kg/kg)	R^2	$1/k_2\ 10^3$ (kg/kg)	R^2
Apple	0.20 ± 0.01	0.984	0.15 ± 0.01	0.994	3.49 ± 0.35	0.960
Banana	0.14 ± 0.03	0.880	0.21 ± 0.01	0.990	2.31 ± 0.40	0.890
Potato	0.14 ± 0.01	0.989	0.08 ± 0.01	1.000	4.67 ± 0.16	0.974

*n.s.= non significant because P>0.05.

The highest rate of water transport observed during the OT of banana occurred with sucrose in the osmotic solution (OS) (moisture $D_e = 4.66\ 10^{-10}\ m^2/s$). The next highest occurred with the sodium chloride in the OS during the OT of potato (moisture $D_e = 1.91\ 10^{-10}\ m^2/s$) and with sucrose in the OS during the OT of apple: in both cases moisture D_e was $1.91\ 10^{-10}\ m^2/s$. The values of the effective moisture diffusivity are in good agreement with the literature (Hough et al., 1993). For instance, Kaymak-Ertekin (2000) calculated D_{ew} values between 10^{-10} and $10^{-11}\ m^2/s$ for apple samples osmotically treated at different temperatures. The results for mass transfer of water are comparable to those found in the literature for potato osmo-treated with sodium chloride OS (Khin et al., 2006).

As far as the mass transfer of solutes is concerned, the structure of the plant tissue and the kind of osmo-active solute play a key role. Peleg constants were used to compare the rate of solute penetration in the several food commodities since the progress of total phenolic content in potato did not fit significantly the diffusional model. During OT with sucrose, the initial rate of phenolic mass transfer of apple (total phenolic $1/k_1$ = 3.02

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10^{-7} s^{-1}) was almost twice higher than that of banana (total phenolic $1/k_1 = 1.73\ 10^{-7}$ s^{-1}). However, OT of potato with NaCl showed the highest initial rate of total phenolic mass transfer (total phenolic $1/k_1 = 7.38\ 10^{-7}$ s^{-1}). Furthermore the penetration rate of osmo-active solute did not follow the same pattern as the rate of total phenolic mass transfer: the highest initial rate of osmo-active solute was during OT of banana with sucrose OS (sucrose $1/k_1 = 5.86\ 10^{-5}$ s^{-1}), followed by that of apple (sucrose $1/k_1 = 4.46\ 10^{-5}$ s^{-1}). The lowest initial rate was found during OT of potato with sodium chloride (NaCl $1/k_1 = 2.73\ 10^{-5}$ s^{-1}).

Results on solute mass transfer during OT strongly depended on the kind of solute and structure of plant tissue. In particular, the resistance to penetration of grape phenolics, which are present in low concentrations compared to osmo-active solutes, is mainly determined by both plant tissue and nature of the osmo-active solute.

7.3.3. Air drying of osmo-treated foods: moisture mass transfer and stability of grape phenolics

Total and individual phenolic content and TEAC were determined before and after air drying (AD) to investigate how AD affects the stability of grape phenolics infused into apple, banana, potato and the model food by a previous OT. Temperature during AD was set to 55 ºC because the degradation of some phenolic compounds occurs at temperatures higher than 50 °C (Larrauri et al., 1997). In particular, air drying at temperatures higher than 60 °C is regarded as unfavorable due to the possibility of inducing oxidative condensation or decomposition of thermolabile compounds, such as (+)-catechin (Asami et al., 2003).

Table 7.3. Effective diffusion coefficients of moisture during air-drying (AD) of osmo-treated apple, banana and potato. Moisture content in dry basis of foods before (after 8h of OT) and after air drying

	$De_W^{AD}\ 10^{10}$ (m^2/s)	R^2	Moisture content before AD (kg/kg dry matter)	Moisture content after AD (kg/kg dry matter)
Gel Sucrose	7.40 ± 0.40	0.947	1.24 ± 0.02	0.50 ± 0.003
Gel NaCl	12.3 ± 0.74	0.952	5.84 ±0.03	1.02 ± 0.061
Gel Control	10.5 ± 0.73	0.935	19.2 ± 0.08	0.23 ± 0.003
Apple Sucrose	9.46 ± 0.33	0.977	2.32 ± 0.03	0.28 ± 0.008
Banana Sucrose	8.45 ± 0.43	0.955	1.76 ± 0.02	0.58 ± 0.014
Potato NaCl	13.3 ± 0.65	0.967	2.11 ± 0.03	0.22 ± 0.031

Experimental and predicted moisture ratio curves are shown in Figure 7.4 to better illustrate dehydration kinetics during AD. Table 7.3 presents initial (i.e. after 8h of OT)

and final moisture contents and the effective diffusion coefficients of moisture during AD (D_{eW}^{AD}). The highest values of D_{eW}^{AD} were 13.3 10^{-10} m^2/s and 12.3 10^{-10} m^2/s, respectively, for potato and model food which had been osmo-treated with sodium chloride OS. Apple and banana osmo-treated with sucrose OS and the model food osmo-treated with non osmo-active solute in OS (control OS) showed D_{eW}^{AD} values of 9.46 10^{-10} m^2/s, 8.45 10^{-10} m^2/s and 10.5 10^{-10} m^2/s, respectively. The slowest drying kinetics during AD were observed for the model food osmo-treated with sucrose OS, (D_{eW}^{AD} =1.01 10^{-9} m^2/s).

a) b)

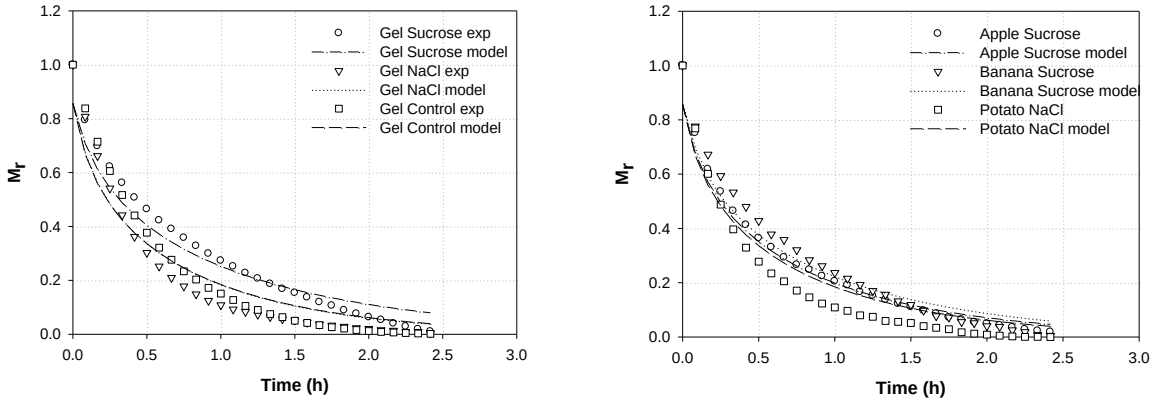

Figure 7.4. Moisture ratio during AD of osmo-treated model food, apple, banana, and potato. Lines are the fitted model.

The slowest drying kinetics during AD were observed for the model food osmo-treated with sucrose OS, (D_{eW}^{AD} =1.01 10^{-9} m^2/s).

In the case of foods osmo-treated with sucrose OS, it has been suggested that the high extent of water loss observed during OT, which led to low moisture contents before AD, hinders further water removal during AD (Torreggiani, 1995). However, AD of potato osmo-treated with sodium chloride OS was significantly faster, and furthermore the initial moisture content of potato after OT was similar to the moisture content of apple after OT with sucrose OS. According to this, other parameters such as the type of osmo-active solute used during OT and the food structure affect water mass transfer during AD.

In the literature, D_{eW}^{AD} values have been reported for osmo-treated apple cubes (1.3 to 3.1 10^{-9} m^2/s) (Prothon et al., 2001; Simal et al., 1997), which were found to be lower than those shown by the non-treated tissue. It has been suggested that the presence of

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sucrose inside and on the surface of the osmo-treated tissue increases the internal resistance to moisture movement during OT (Rahman et al., 1991).

The total phenolic content (dry basis) and TEAC (dry basis) values of fresh, osmo-treated and osmo-air-dried samples (Figure 7.5) were determined to analyze how air drying affects the stability of the grape phenolics infused into the food by OT. The reduction (%), *R*, in the total phenolic content and the TEAC was calculated as follows:

$$R^{TPH}(\%) = \frac{x_{AD_0}^{TPH} - x_{AD_f}^{TPH}}{x_{AD_0}^{TPH}} \times 100 \qquad (7.9)$$

$$R^{TEAC}(\%) = \frac{TEAC_{AD_0} - TEAC_{AD_f}}{TEAC_{AD_0}} \times 100 \qquad (7.10)$$

where subindex AD_0 and AD_f represent initial and final conditions during AD and superindex TPH indicates total phenolics.

The model food osmo-treated with the control OS (i.e. with non osmo-active solute) showed the highest reduction in total phenolics (9.0%) followed by osmo-treated banana (3.7%) and apple (3.7%). OT of the model food with sucrose and sodium chloride OSs led to an increase in the total phenolic content (negative values of reduction (%)), which was also observed in potato to a lesser extent.

TEAC in the model food followed a similar trend as that described for total phenolic reduction. There was high TEAC reduction (14.4%) in samples osmo-treated with the control OS and an increase of TEAC in samples osmo-treated with sucrose and sodium chloride OSs. However, the total phenolics increased about twice as much as TEAC in model food osmo-treated with sucrose and sodium chloride OSs. Unlike the total phenolic reduction observed in apple and banana, TEAC showed an important increase of 24.0% and 66.6%, respectively. In potato, TEAC increase was about two times greater than that of total phenolics. Previous studies on the stability of phenolics during AD of olive mill wastes (Obied et al., 2008) and prunes (Piga et al., 2003) have shown a reduction in the total phenolic content and a minor reduction or even an increase in antioxidant capacity depending on the AD conditions. Different reaction pathways have been suggested to explain theses results: i) phenolic oxidation that results in intermediate compounds with great antioxidant activity, even though this is temporary, ii) thermal degradation of some large molecular weight phenolics that can generate

more active small molecular antioxidants, and iii) release of bound phenolic acids or bound phenolics compounds (Maillard et al., 1995).

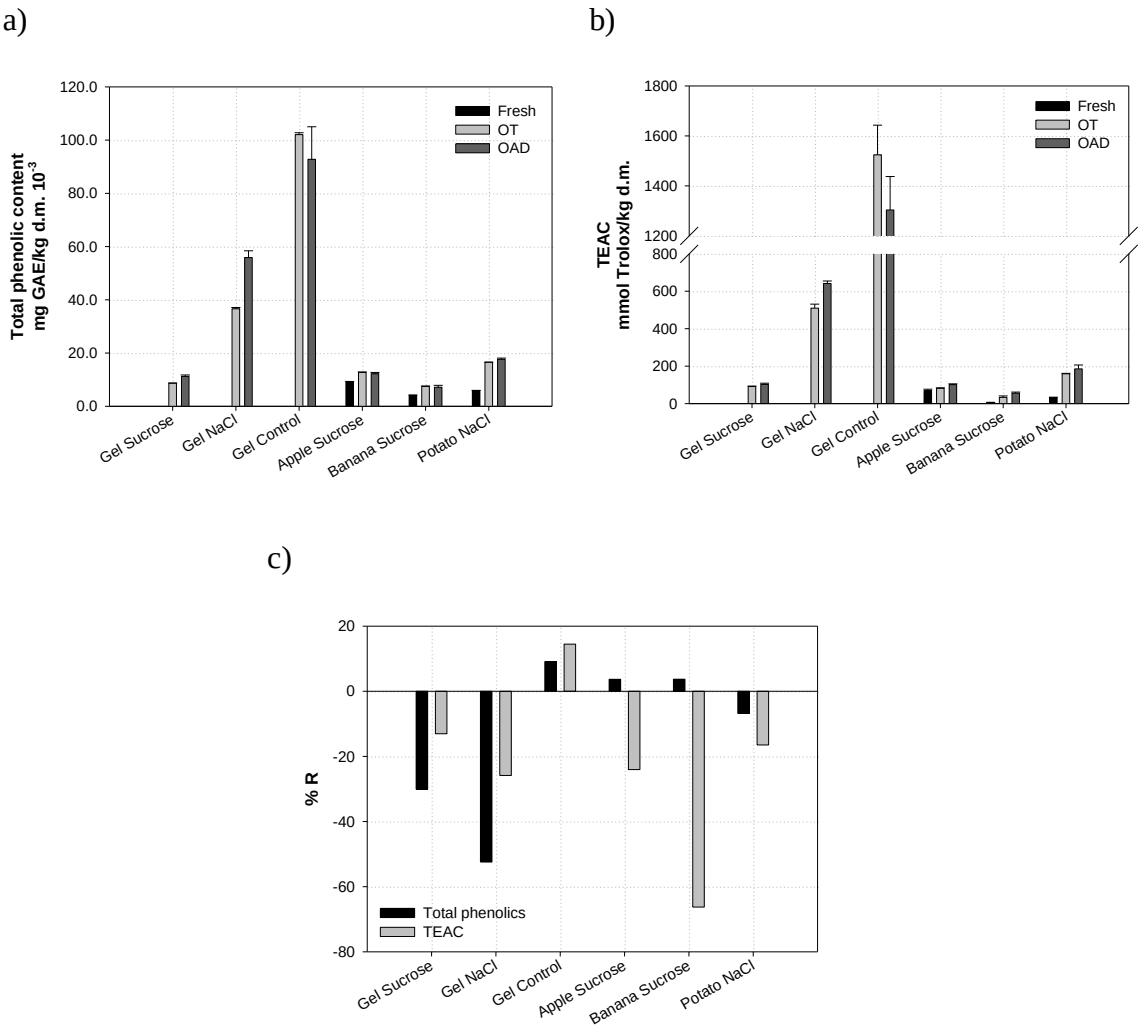

Figure 7.5. Total phenolic content (a), and TEAC (b) of fresh, osmo-treated (OT) and osmo-air-dried (OAD) food. Reduction (%) in total phenolic content and TEAC of osmo-treated and osmo-air-dried food (c).

Considering all this, the food composition and structure of apple, banana and potato, which determine the chemical and biochemical activity of all three, strongly affected changes in both total phenolic content and TEAC during AD. In banana and apple, OT with sucrose OS led to a small reduction in the total phenolics and a huge increase in TEAC, while the same OT of the model food caused an important increase in the total phenolic content but a lower increase in TEAC than that of apple and banana (Figure 7.5). In the case of the model food osmo-treated with sodium chloride OS, total phenolic content and TEAC strongly increased during AD.

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To better explain such behavior, individual grape phenolics of low molecular weight (below 600g/mol) were determined in the model food before (i.e. after 8h of OT) and after AD. Specifically, the hydroxybenzoic acids, flavan-3-ol monomers and dimers shown in Figure 7.6 were quantified. The reduction (%) during AD of each of these individual phenolics was calculated as follows:

$$R^{PHj}(\%) = \frac{x_{AD_0}^{PHj} - x_{AD_f}^{PHj}}{x_{AD_0}^{PHj}} \times 100 \quad (7.11)$$

where subindex AD_0 and AD_f represent initial and final conditions during AD and superindex PHj indicates each individual phenolic identified.

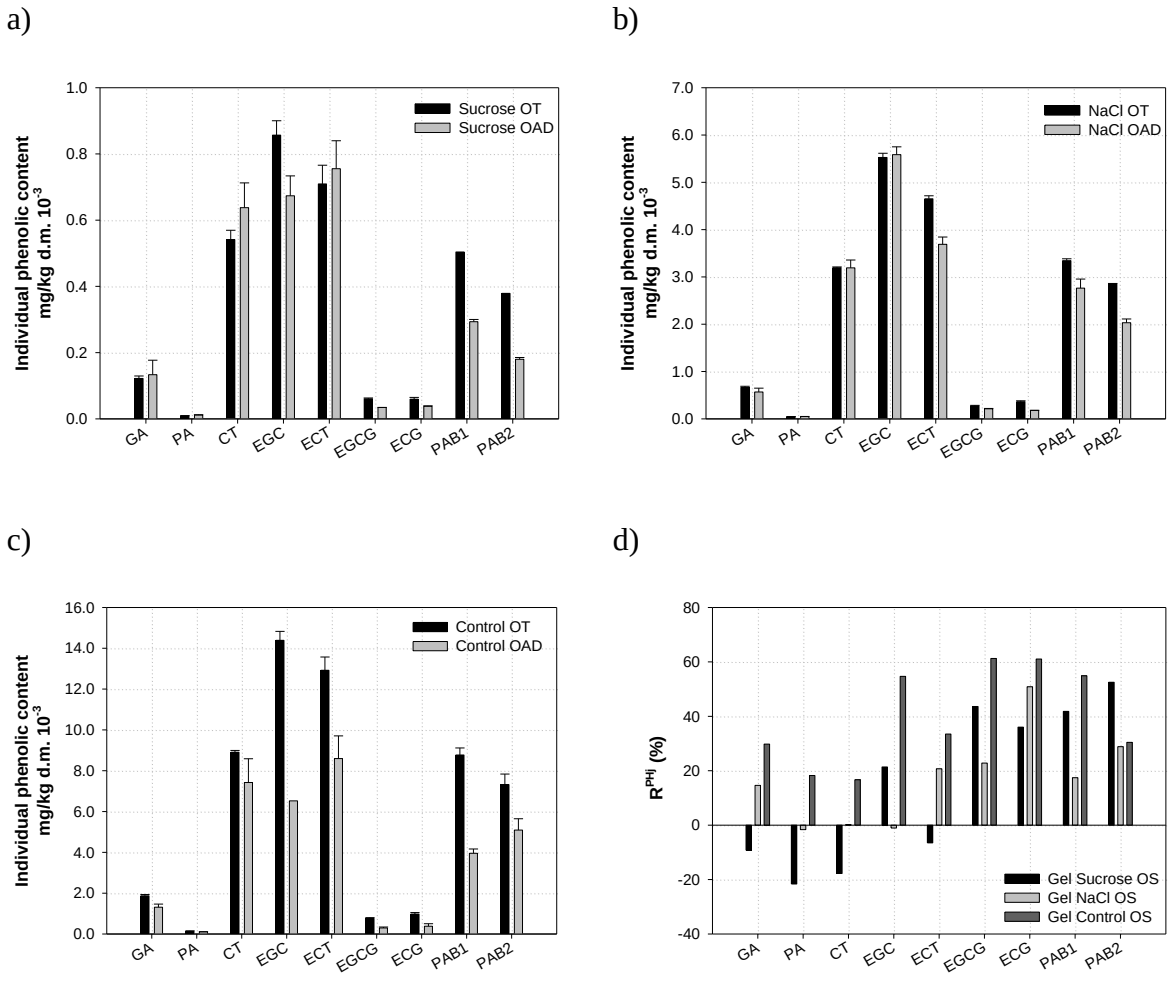

Figure 7.6. Individual phenolic content in osmo-treated (OT) and osmo-air-dried (OAD) model food. OT was performed with sucrose (a), sodium chloride (b), and control, with non osmo-active solute, (c) OSs. Reduction in individual phenolic content during AD (d).

OT with the control OS led to the highest reduction in all the phenolics analyzed upon AD, with maximum values around 60% for EGCG and ECG (Figure 7.6d). The model food osmo-treated with sucrose in OS showed a different behavior during AD. The content of flavan-3-ol dimers, such as PAB1 (R^{PAB1} = 41%) and PAB2 (R^{PAB2} = 52%), significantly decreased while GA (R^{GA} = -10%), PA (R^{PA} = -22%), CT (R^{CT} = -12%) and ECT (R^{ECT} = -7%) content notably increased. In the case of AD of model food osmo-treated with NaCl OS, there was a significant reduction of all individual phenolics analysed except PA and EGC which showed a slight increase in their content.

Overall, these results suggest that the sucrose and sodium chloride infused into the osmo-treated model food protected against phenolic degradation. A similar effect has been observed during frozen storage of kiwi fruit and strawberry: a previous OT with sugars increased the retention of chlorophyll and vitamin C in kiwi fruit (Torreggiani et al., 2001) and of pigments in strawberry (Torreggiani et al., 1995).

A detailed description of how sucrose and sodium chloride can protect against phenolic degradation requires thorough research into the numerous chemical pathways involved. Nevertheless, because there is a simultaneous reduction in flavan-3-ol dimers and an increase in hydroxybenzoic acids, (+)-catechin, and epicatechin after AD of model food osmo-treated with sucrose OS (Figure 7d) some of these mechanisms may involve the thermal degradation of polymerized phenolics and/or the release of bound phenolics.

7.4. CONCLUSIONS

The total phenolic content and antiradical scavenging capacity of plant foods such as apple, banana, and potato were significantly increased by OT with a grape seed extract as a source of phenolics. In this sense, the extent of grape phenolic impregnation was controlled by food structure and the kind of osmo-active solute. Overall, plant tissue showed a lower grape phenolic infusion than that of a model food made of agar gel.

OT, as a pre-treatment, seemed to protect against grape phenolic degradation during further AD, although the mechanisms controlling the process require further research.

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1. Rózek Aleksandra, Achaerandio Isabel, Almajano María Pilar, Güell Carme, López Francisco, Ferrando Montserrat. **(2007).** Solid foodstuff supplemented with phenolics from grape: antioxidant properties and correlation with phenolic profiles. ***Journal of Agricultural and Food Chemistry*,** 55, 13, 5147-5155.
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2. Rózek, A.; Achaerandio, I.; Güell, C.; López, F.; Ferrando, M. (**2007**). Mass Transfer during osmotic dehydration in a multicomponent solution rich in grape polyphenols with high antioxidant activity. ***Drying Technology***, 25, 11, 1234-1245.
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3. Rózek, A., Achaerandio I, Güell C, López F, Ferrando M. (**2008**) Direct formulation of a solid foodstuff with phenolic-rich multicomponent solutions from grape seed: effects on composition and antioxidant properties. ***Journal of Agricultural and Food Chemistry***, 56, 12, 4564-4576.
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4. Rózek, A., Achaerandio, I., Güell, C., López, F., Ferrando, M. (**2008**). Grape phenolic impregnation by osmotic treatment: influence of osmotic agent on mass transfer and product characteristics. ***Journal of Food Engineering*.** Submitted
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5. Rózek, A., Achaerandio, I., Güell, C., López, F., Ferrando, M. (**2008**). Effect of air drying on the stability of grape phenolics infused into fruits and vegetables by osmotic treatment. ***Food Research International*.** Submitted
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6. Rózek, A., Achaerandio, I., Güell, C., López, F., Ferrando, M. (**2008**). Use of commercial grape phenolic extracts to supplement solid foodstuff. ***Journal of Food Engineering***. Submitted
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"I have not eaten enough of the tree of knowledge,
though in my profession I am obligated to feed on it regularly".

Albert Einstein

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