UNIVERSIDADE DA BEIRA INTERIOR
Ciências

Peach from Cova da Beira as Health Promotors

Catarina Pereira Bento

Dissertação para a obtenção do Grau de Mestre em
Bioquímica
(2º ciclo de estudos)

Orientador: Prof. Doutor Luís Rodrigues da Silva
Co-orientador: Prof. Doutora Branca Monteiro da Silva

Covilhã, outubro de 2016

Aknowledgements

I would like to start by thanking to Professor Luís Rodrigues da Silva for believing in my capabilities, his endless efforts during this challenge and by always being there to clarify my doubts. This work would've not been possible without his guidance and corrections.

I thank to Professor Branca Silva for her supervision and enriching contribute to the development of this dissertation and correspondent correction.

A very special thank you to Ana Raquel Nunes for being so patient and making herself available to introduce me and my colleague to the Centro de Investigação em Ciências da Saúde (CICS) facilities and guiding us through the initial part of our projects. Without her it would have been so much harder.

I thank the Centro de Investigação em Ciências da Saúde (CICS) for providing all the equipment necessary. Also thanking the Centro Hospitalar Cova da Beira, E.P.E. for their collaboration in the development of the experimental work providing the necessary blood samples, and to Cerfundão for providing all the peach samples.

To my dear colleague Ana Carolina Gonçalves for her help and for being my friend, confident and always brightening my days, no matter how bad they were.

To all my friends for their support, short or long distance. My new colleagues from the Biochemistry Master's class. My new friends in Covilhã who have been a great emotional support. Cavendish and Five to One for their friendship and musical therapy sessions. A special thank you to Daniela Pacheco for her endless patience to listen, encouragement and love.

I thank my godmother Augusta Cerca for always being there, for her care and support.

Finally and most importantly, I thank my parents Maria Armanda and Abel for literally everything! Without them this journey would have been impossible.

Abstract

The peach's chemical constitution comprises various antioxidant compounds such as phenolic compounds (flavonoids and non-flavonoids) carotenoids and vitamin C. Their properties have been object of investigation in many areas, proving to have preventive and protective effects against many chronic and age-related diseases such as diabetes, obesity, hypertension, inflammation, cardiovascular, neurodegenerative and oncologic diseases. In this work we evaluate the phenolic profile and biological potential of six peach cultivars (*Summer Rich*, *Fidelia*, *Royal Glory*, *Royal Magister*, *Royal Lu* and *Sweet Dreams*) from Fundão region (Portugal), thus establishing its potential role as a health promotor. The LC-DAD analysis revealed a total of 3 anthocyanins with cyanidin-3-*O*-glucoside as the main one; and 14 non-colored phenolic compounds which include one hydroxybenzoic acid, eight hydroxycinnamic acids, three flavan-3-ols and three flavonol, chlorogenic and neo-chlorogenic acids are the most abundant, as well as some catechin derivatives. The antioxidant potential was evaluated by FRAP assay and against DPPH and NO radicals. The *in vitro* assays revealed a good antioxidant activity. *Fidelia* (13.8 μM Fe^{2+}) presented the major antioxidant power in FRAP, *Royal Lu* proved to be the most active against DPPH• (IC_{50} = 62.1 ±1.5 μg/mL), and *Sweet Dreams* was the most active against NO radical (IC_{50} = 421.2 ± 45.4 μg/mL). Regarding the α-glucosidase inhibitory activity, Royal Magister and Royal Glory present the highest activity (IC_{50} = 11.7 ± 1.4 and 17.1 ± 1.7 μg/mL, respectively). Finally, the protective effect of *Royal Lu* to prevent oxidative damage in human erythrocytes induced by ROO• was performed. *Royal Lu* proved to be quite efficient with promising results, showing an IC_{50} value of 110.0 ± 4.5 μg/mL and IC_{50} at 83.8 ± 6.5 μg/mL for hemolysis and hemoglobin oxidation inhibition. Thus, peach may be considered a promising source of bioactive compounds to be used by the food and pharmaceutical industries, once the inhibition of oxidative mechanisms by antioxidant compounds is a fundamental property that can reduce oxidative stress associated with various diseases.

Keywords

Peach; Phenolic compounds; Antioxidant activity; Antidiabetic potential; Antihemolitic potential.

Resumo

A composição química do pêssego incorpora vários compostos com propriedades antioxidantes, tais como compostos fenólicos (flavonóides e não-flavonóides), carotenóides e vitamina C. Tais propriedades têm sido alvo de investigação em diversas áreas da saúde, demonstrando possuir efeitos protectores e preventivos contra doenças crónicas como a diabetes, obesidade, hipertensão, inflamação, doenças cardiovasculares, neurodegenerativas e oncológicas. No trabalho desenvolvido nesta tese de mestrado avaliou-se o perfil fenólico e o potencial biológico de seis variedades de pêssego do Fundão (*Summer Rich*, *Fidelia*, *Royal Glory*, *Royal Magister*, *Royal Lu* e *Sweet Dreams*), estabelecendo desta forma o seu potencial papel como promotor da saúde. A análise por LC-DAD permitiu a identificação de 3 antocianinas, sendo a cianidina-3-*O*-glucósido o composto maioritário; e 14 compostos fenólicos não corados, os quais incluem 1 ácido hidroxibenzóico, 8 ácidos hidroxicinâmicos, 3 flavan-3-óis e 3 flavonóis, sendo os ácidos clorogénico e neoclorogénico os compostos mais abundantes, assim como alguns derivados da catequina. O potencial antioxidante foi avaliado através do FRAP e da capacidade de intersecção dos radicais DPPH e NO. Os ensaios *in vitro* demonstraram uma boa capacidade antioxidante. *Fidelia* (13.8 µM Fe^{2+}) apresentou um maior poder antioxidante no FRAP, a variedade *Royal Lu* mostrou ser a mais ativa contra o DPPH• (IC_{50} = 62,1 ±1,5 µg/mL), a *Sweet Dreams* (421,2 ± 45,4 µg/mL) foi a variedade que apresentou maior capacidade de interseção do radica NO. Relativamente à inibição da α-glucosidase, as variedades *Royal Magister* and *Royal Glory* apresentaram-se como as mais ativas (IC_{50} = 11,7 ± 1,4 e IC_{50} = 17,1 ± 1,7 µg/mL, respectivamente). Finalmente, o efeito protector da variedade *Royal Lu* contra o dano oxidativo induzido por radicais peroxilo em eritrócitos humanos foi avaliado. Os resultados demonstraram uma actividade dependente da concentração, com IC_{50} de 110,0 ± 4,5 µg/mL para a inibição da hemólise e IC_{50} at 83,8 ± 6,5 na oxidação da hemoglobina. O pêssego pode ser considerado uma fonte promissora de compostos bioativos que poderão ser usados pelas indústrias alimentares e farmacêuticas, uma vez que a inibição de mecanismos oxidativos através de compostos antioxidantes é uma propriedade fundamental que poderá reduzir o stress oxidativo associado a várias doenças.

Palavras-chave

Pêssego; Compostos fenólicos; Atividade antioxidante; Potencial anti-diabético; Potencial anti-hemolítico.

Resumo Alargado

Nos últimos anos os frutos e vegetais têm sido alvo de grande interesse por parte da população e comunidade científica, devido aos seus inúmeros benefícios na saúde humana, já comprovados através de vários estudos científicos, conferindo proteção contra várias patologias, tais como doenças cardiovasculares, neurodegenerativas, oncológicas e inflamatórias.

O pêssego (*Prunus persica* (L.) Batsch) pertencente à familia Rosaceae teve a sua origem na China e é um dos frutos mais consumidos a nível mundial. As diversas variedades de pêssego diferem em forma, tamanho, cor e textura da polpa (branca e amarela), tipos de casca e caroço (aderente ou não aderente à polpa). Este fruto é consideravelmente rico em antioxidantes, sendo uma importante fonte de vitaminas A, B e C, carotenóides e compostos fenólicos, como os ácidos 3-*O*-cafeoilquínico e 5-*O*-cafeoilquínico, catequinas, quercetinas e cianidinas. A sua composição química varia de acordo com a variedade, condições edafo-climáticas, práticas agrícolas, entre outras. Ultimamente os compostos fenólicos têm sido o centro de atenção por parte da comunidade científica devido às suas imensas propriedades bioativas, sendo por isso de grande interesse explorar a constituição e propriedades biológicas do pêssego.

Com este estudo pretendeu-se determinar e avaliar o perfil fenólico de seis variedades diferentes de pêssego (*Summer Rich*, *Fidelia*, *Royal Glory*, *Royal Magister*, *Royal Lu* and *Sweet Dreams*) da região do Fundão, assim como avaliar o seu potencial biológico. Para tal procedeu-se à preparação de extratos hidroethanólicos através de coluna sep-pak C18 para determinação das antocianinas e fenóis não corados, e realização de vários ensaios *in vitro* de forma a testar a atividade antioxidante, atividade inibitória da enzima α-glucosidase e a sua capacidade protetora contra o dano oxidativo de eritrócitos humanos.

A avaliação do perfil fenólico através do LC-DAD permitiu a identificação e quantificação de dezassete compostos fenólicos, que englobam três antocianinas e catorze compostos não corados. Em relação às antocianinas, o conteúdo total variou entre 2,6 µg/g e 153,4 µg/g expresso em fruto seco, salientando a cianidina-3-*O*-glucósido como o composto maioritário em todas as variedades, oscilando entre 55,6% e 91,2% do conteúdo total de antocianinas. A *Royal Magister* revelou ser a variedade mais rica em antocianinas. No que toca a compostos não-corados, os conteúdos totais variaram entre 221.01 µg/g a 1287.8 µg/g expresso em fruto seco. Os ácidos 3-*O*-cafeoilquínico e 5-*O*-cafeoilquínico foram os compostos predominantes nas variedades *Fidelia*, *Royal Magister* e *Sweet Dreams*, representando 6,46% a 40,09% do total do conteúdo de compostos fenólicos não corados. *Royal Lu* e *Sweet Dreams* apresentaram o derivado do ácido benzóico como o composto maioritário, com teores que

oscilaram entre 0s 58,3-88,7% do total em compostos fenólicos não corados. Finalmente, a variedade *Royal Glory* apresentou o derivado de catequina 1 como o composto maioritário (49,6%). Esta última variedade foi a mais rica em compostos fenólicos não corados.

Os radicais livres (ROS e RNS) estão implicados em vários processos biólogos, no entanto a produção excessiva ou fraca eliminação dos mesmos pelos mecanismos naturais pode levar a danos celulares e ao desenvolvimento de doenças devido ao stress oxidativo. Os compostos fenólicos possuem estruturas químicas únicas que lhes conferem potencial redutor, atuando como antioxidantes eliminando os radicais livres. A atividade dos extratos de pêssego foi avaliada através dos seguintes ensaios: FRAP, atividade contra os radicais DPPH e óxido nítrico. As variedades que mais se destacaram no FRAP foram a *Royal Glory* (12,8 µM Fe^{2+}), *Fidelia* (13,8 µM Fe^{2+}) e a *Royal Lu* (13,9 µM Fe^{2+}). No caso do ensaio do DPPH•, as variedades mais ativas foram a *Royal Lu* (IC_{50} de 62,1 ±1,5 µg/mL) e a *Sweet Dreams* (65,1 ± 2,5 µg/mL), esta última também mostrou ser a mais ativa contra o óxido nítrico (IC_{50} = 421,2 ± 45,5 µg/mL). Esta atividade antioxidante está associada ao seu alto conteúdo em ácidos fenólicos e demais flavonóides, cuja estrutura química permite facilmente ceder átomos de hidrogénio.

A *diabetes mellitus* é um grupo de perturbações metabólicas, caraterizada por hiperglicemia e distúrbios no metabolismo dos hidratos de carbono, gorduras e proteínas. Esta hiperglicémia traz consequências e danos a longo prazo, danos funcionais e falência de vários órgãos. A enzima α-glucosidase tem sido um alvo terapêutico, dado o seu papel na digestão dos hidratos de carbono, a sua inibição é fundamental para a quebra dos hidratos de carbono complexos em monossacarídeos absorvíveis, de forma a baixar o índice glicémico e o desenvolvimento da diabetes. Todos os extratos mostraram boa atividade inibitória, superando a acarbose que foi usada como controlo positivo. As variedades mais ativas foram a *Royal Magister* (IC_{50} = 11,7 ± 1,4 µg/mL) e *Royal Glory* (IC_{50} = 15,0 ± 1,2 µg/mL), sendo também as duas variedades mais ricas em antocianinas.

As membranas eritrocitárias são estruturas bastante ricas em ácidos gordos, logo bastante suscetíveis à peroxidação lipídica mediada por radicais livres. Isto pode acontecer tanto devido ao transporte de oxigénio como um desequilíbrio entre elementos oxidantes e antioxidantes. Através de uma simulação de ambiente oxidativo com radicais peroxilo foi possível avaliar a atividade do extrato de pêssego *Royal Lu* que inibiu com sucesso a hemólise das células (IC_{50} =110.0 ± 4,5 µg/mL) e a oxidação da hemoglobina (IC_{50} = 83,8 ± 6,5 µg/mL). Esta atividade está associada com a riqueza desta variedade em compostos fenólicos, capacidade de eliminar os radicais peroxilo e à sua influência na bicamada lipídica da membrana, diminuindo a sua fluidez e impedindo a difusão de radicais livres através da mesma e os consequentes danos.

Estes resultados revelaram a qualidade do perfil fenólico do pêssego. Os ácidos hidroxicinâmicos possuem uma elevada atividade antioxidante devido à sua estrutura CH=CH-

COOH, resultando num maior potencial redox, cedendo facilmente átomos de hidrogénio. Adicionalmente, os flavonóides colaboram também com grande poder antioxidante devido às características chave da sua estrutura: grupo catecol no anel B, um grupo 4-oxo e a ligação dupla C2=C3 no anel C. Estes compostos e as suas caraterísticas estruturais únicas conferem ao pêssego a sua atividade biológica, no entanto não poderemos descartar a presença de outros compostos bioativos que não foram identificados. Tais constituintes são importantes elementos na integridade do fruto, sendo também um fator-chave responsável pelo poder antioxidante do pêssego.

Em forma de conclusão, poderemos dizer que os pêssegos são uma excelente fonte de fitoquímicos e antioxidantes naturais e que devem ser incluídos nos frutos preferenciais dos consumidores, sendo recomendado o seu consumo diário.

Index

Lista de Figuras

Lista de Tabelas

...

Acronym list

AAPH	2,2′- azobis (2-ethylpropionamidine) and dihydrochloride
AChE	Acetylcholinesterase
ATP	Adenosine Triphosphate
BChE	Butyrylcholinesterase
DM	*Diabetes mellitus*
DNA	Desoxyribonucleic Acid
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EGFR	Epidermal Growth Factor Receptor
eNOS	Endothelial nitric oxide synthase
GABA	y-aminobutyric acid
GPx	Glutathione peroxidase
GR	Glutathione reductase
HDL	High density lipoprotein
HPLC	High pressure liquid chromatography
iNOS	Inducible nitric oxide synthase
LC/DAD	Liquid chromatography/diode array detector
LDL	Low density lipoprotein
LOD	Limit of detection
LOQ	Limit of quantification
MLC	Myosin light chain
MYPT1	Myosin phosphatase target subunit 1
NADH	ß-nicotinamide adenine dinucleotide
NAD(P)H	Nicotinamide adenine dinucleotide phosphatase
NBT	Nitrotetrazolium blue chloride
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
P_i	Inorganic phosphorus

PBS	Phosphate-buffered saline
SNP	Sodium nitroprusside dihydrate
TA	Superoxide dismutase
TEAC	Trolox equivalent antioxidant activity
TNF-α	Tumor necrosis factor-α
USDA	United States Department of Agriculture
UV	Ultra Violet
WHO	World Health Organization

1. Introduction

1.1. The Peach

It is known nowadays through various epidemiological studies that a diet rich in vegetables and fruits brings many health benefits like protection against chronic diseases, such as cardiovascular and neurodegenerative diseases, cancer and many other health compromising conditions (1–3). The World Health Organization (WHO) has defined vegetables as edible plants with nutritional value or, from a botanical point of view, as the "edible part of a plant", which would make fruits a subset of vegetables. Fruit is the plant's mature ovary containing the seeds (4). Besides this, vegetables and fruits are known to be functional foods (5). Both are low energy-dense foods and have a high content of bioactive compounds, as well as fiber, vitamins and water (4,5).

The peach (*Prunus persica* (L.) Batsch) (Figure 1) is a fruit that belongs to the *Rosaceae* family and its origin dates back to 1100 B.C. in China (6). It is one of the most variable species of fruit assuming different shapes, sizes, flesh (white or yellow flesh), types of skin, the seed, and the flower can also vary in size and colour, among other variable aspects regarding this popular fruit (7). It can be divided into two categories, freestone and clingstone, referring to the adherence of flesh to the stone. Both can be of white or yellow flesh according to mesocarp colour (6). The anatomy of peach is represented in Figure 2.

Figure 1. *Prunus persica* (L.) Batsch

This fruit is considerably rich in antioxidants, being an important source of vitamins A, B and C, carotenoids and phenolic compounds like chlorogenic and neochlorogenic acids, catechin, epicatechin, cyanidin and quercetin derivatives (6,8–10). These phytochemicals are not uniformly distributed in the fruit tissue They have been found to be concentrated in the peel, more specifically the epidermal and subepidermal layers (5,8,10). In sum, peel contains higher amounts of phenolic compounds and other antioxidants than flesh tissue, supporting the attributed protective role (10,11). Regarding chemical composition and relative quantities, they are influenced by several factors such as genotype, geographic and climacteric conditions, seasonal and weather conditions, agronomic practices, maturity stage, storage conditions and processing methods (12).

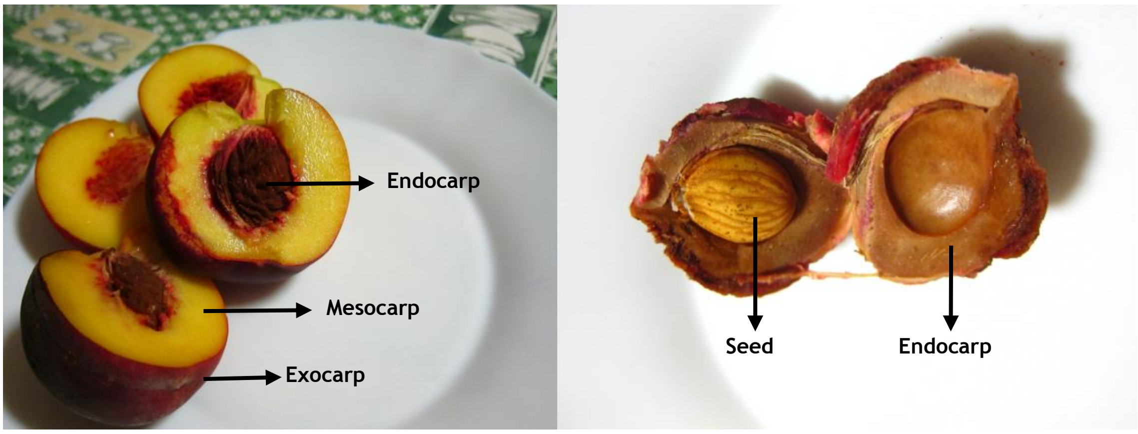


Figure 2. The anatomy of peach fruit.

Being a worldwide consumed fruit it is of our interest to include it in further studies. Peach cultivars have been classified according to their phenolic profile (13). This may help determine how a certain cultivar influences our health through the bioactive properties of phenolic compounds that have long been the centre of attention on several studies due to their antioxidant, antimicrobial and anti-inflammatory properties.

1.1.1. General chemical composition

The chemical composition of fruits is an important quality determinant, it influences nutritional and health benefits, taste and aromas. Like other fruits, the peach's nutritional value and quality is determined by its chemical composition (14–16). Most quality evaluations focus on sugar/acid ratio content. This is assessed by the soluble solid content (Brix°) and titratable acidity (TA) (17). Brix° values in peach vary from 9.55 to 19.83, and TA between 2.17 and 12.07 g of malic acid L^{-1} (18). The peach's approximate chemical composition is enlisted in Table 1, and these values may vary according to the cultivar.

1.1.2. Carbohydrates

Carbohydrates are the most abundant macronutrients in fruits and vegetables, counting 50 to 80% of their dry weight (19). They all represent an important source of energy during fruit ripening (19,20). More importantly, they are the main source of energy in the human diet and also play a role regulating the intestinal microflora (21).

Carbohydrates can be divided into 3 groups: monosaccharides which include pentoses (arabinose, xylose, ribose) and hexoses (glucose, fructose, galactose), oligosaccharides (sucrose, maltose, lactose, raffinose) and polysaccharides (starch, glycogen) (21). The monosaccharides and oligosaccharides are very important in defining organoleptic characteristics like taste and aroma (14,15,21). The sugar profile determines traits such as flavour, texture and health properties (9).

The main sugars present in peach are sucrose, fructose, sorbitol and glucose, representing around 8.3% of fresh fruit (Table 1) (14,15). According to a study of 106 peaches from different cultivars, the total amount of sugars in peaches varies from 89.16 – 184.49 g/L: sucrose representing 55.74% - 72.96%, glucose 6.65% - 15.42%, fructose 6.77% - 16.82% and sorbitol 1.07% – 10.75% (18). The proportions of these sugars undergo changes as the fruit matures (20,21). Their levels tend to increase and they continue to increase also after the peach is harvested until it reaches the ideal point to be eaten. Along with sugar increase new aromas and tastes develop and acid concentrations decrease. Sucrose, glucose and fructose contents increase are a result of long-chain carbohydrates hydrolysis in high energy metabolism processes (20). Sucrose and sorbitol are two of the main sugars found in peach that play a key role in aroma. Peaches with higher amounts of these sugars, most importantly sucrose, have better aromas (14,20). Besides being a sweetener, sucrose plays an important role as an energy source. Sorbitol is a sugar alcohol that can act as a laxative in the body since it promotes osmotic transfer of water into the bowel (21). There is an increasing interest in this sugar since its alcohol possesses more benefits like dental health and gastrointestinal problems, moreover, it can be used as an alternative to glucose for diabetics (9). Glucose and fructose are reducing sugars and are present in higher amounts in early stages of maturation, being gradually surpassed by sucrose during the fruit ripening (19,20). Fructose has a higher level of sweetness than sucrose and glucose which makes it an important influence in taste. It has also been found to have gastrointestinal benefits because it induces bifidobacteria and lactobacilli growth in the gastrointestinal tract (9).

Qualities like aroma do not depend only on the sugars. The balance between sugars and organic acids is an important quality factor for the peach and other fruits, contributing to their sensorial characteristics (14,15).

1.1.3. Fiber

The peach's peel is very rich in both soluble and insoluble fibres, making unpeeled peaches a good fiber source — 1.5 g per 100 g of fruit (Table 1). Their regulatory capacity in the gastrointestinal and circulatory systems helps in weight control and maintaining low cholesterol levels. Along with vitamins, it is why fruits are so important in a healthy and balanced nutrition (21,22).

1.1.4. Fatty Acids

Triglycerides, or fats are a great source of energy, however they represent a small part of a fruits' composition (21). According to the USDA, for a 100 g portion of peach the amount of fat is approximately 0,25 g, as shown in Table 1 (23). Still these fatty acids are important as they serve as carriers for some lipophilic vitamins and bioactive compounds present in fruits, and the presence of essential fatty acids in their composition is believed to play an important role in the prevention of cardiovascular diseases (21,24). Some fatty acids are important in cell maintenance since they are precursors of prostaglandins (21). Studies using peach fruit and peach oil have shown the presence of polyunsaturated linoleic and linolenic acids, and saturated palmitic acid as the main fatty acids (24,25). Both linoleic and linolenic acids are not synthetized in the body, being essential fatty acids needed for building and repairing cell walls, tissues in the nervous system and in the formation of prostaglandins. Moreover, polyunsaturated fatty acids seem to play a role in preventing inflammatory and other chronic diseases, and promoting low levels of total cholesterol and high density lipoprotein (HDL) (21,24).

1.1.5. Amino acids

It is important to mention that the amino acid profile of the peach also takes part in the fruit's taste. They can either occur in the free form or bound in proteins and they may increase the taste of other compounds present in the fruit. These amino acids can be grouped in tasteless (arginine, asparagine, isoleucine, lysine, serine, threonine and valine), bitter (leucine, phenylalanine, tryptophan and tyrosine) and sweet amino acids (proline and alanine). All the previously mentioned amino acids are found in peach varying in quantity according to the cultivar (26,27). Among these there are a few which are classified as essential amino acids: arginine, lysine, tyrosine and phenylalanine (26).

Determination of peaches' amino acid content has been assessed using peach juice concentrate (70° Brix) obtained from Roberts variety. The results were expressed in mg/L at 12° Brix (28). In this study, asparagine presented the highest concentration (2491 ± 25 mg/L), followed by aspartic acid (616.4 ± 5.0 mg/L), Serine (370.2 ± 4.1 mg/L) and glutamic acid (283.0 ± 2.8 mg/L) which also represent the majority according to the USDA's database (Table 1). This database features tryptophan (0.010 g), methionine (0.010 g), cystine (0.012 g) histidine (0.013 g) and tyrosine (0.014 g) as the minority amino acids found in peach, and adding to these Buedo et al. (2001) also lists phenylalanine (38.30 ± 0.73 mg/L) and ethanolamine (40.20 ± 0.45 mg/L) (Table 1) Differences between the values obtained may be due to variety differences as well as the type of matrix analysed. Other amino acids can be found in peach, such as threonine, isoleucine, leucine, lysine, valine, Arginine, alanine, glycine and proline which are enlisted in Table 1, and γ-amino butyric acid (GABA) (23,28).

1.1.6. Organic acids

Sweetness is related to sugars and sourness to organic acids (14,15). Organic acids are low molecular weight compounds consisting of carbon, hydrogen and oxygen atoms and possess one or more carboxyl groups. They are mainly concentrated in the roots of plants and play a part in the nutrient uptake process by plants and microorganisms according to the number of carboxylic groups and their respective dissociation properties (Jones 1998). They are also associated to metal detoxification by plants (Jones 1998). It has been pointed that the ratio of sugars/organic acids is important as it affects organoleptic qualities, in this case, it determines if the fruit is more or less sweet or even sour. This is because there is a negative correlation between sweetness and organic acid concentrations. High concentrations of sugar are easily hidden by high concentrations of organic acids that dim the perception of sweetness. This varies according to the predominant organic acid, for example, citric acid adds more sourness than malic acid, meaning different organic acids require different concentrations to affect sugars perception (14,15). The main organic acids in peach are malic and citric acids. Shikimic, fumaric and quinic acids are also present in smaller concentrations (14,15). In a study conducted by Reig and collaborators, the total acid content measured in peach ranged from 5.59 – 18.50 g/L. This includes: malic (42.92 % – 84.30%), citric (3.72% – 31.61%), quinic (14.56% - 57.54%) and shikimic (0.14% - 1.89%) acids (18).

Taste is another organoleptic trait affected by sugar and organic acid levels. Sucrose seems to represent the major part of sugar content, not only responsible for sweetness, it demonstrates its importance by preventing oxidation reactions. Although, after stating facts one can say that the degree of sweetness is more related to organic acid content (14,15). Sorbitol contributes to taste by improving fruit texture which is very important. The ratio between malic acid and citric acid also influences the taste (14).

1.1.7. Minerals

Minerals can be divided into macroelements and microelements seeing that they play different roles in the plant metabolism. Percentage of total ashes is around 0,6-0,86% (26). Potassium, calcium and magnesium are macroelements present in peach, and these play a key role in the metabolism, participating in the synthesis of amino acids and proteins. According to the USDA and INSA databases, the most abundant mineral in peach was potassium, followed by phosphorus, magnesium and calcium (Table 1) (23,30). As for microelements, they are part of the enzymatic system, iron, copper, zinc and manganese are part of the peach's composition (26). These minerals are also fundamental to human health. The mineral content reported in peach (peel and pulp) consists essentially of: nitrogen (N), phosphorus (P), potassium (K) calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chromium (Cr), nickel (Ni), cobalt (Co), lead (Pb), selenium (Se) and fluoride (F^-) (23,31,32). Potassium is very important in electrolyte balance and cell maintenance (33). The phosphorus in the body is mainly present in the bone tissue, representing around 70%. The other percentage is in form of inorganic phosphorus (Pi) which is also essential in cell maintenance and normal functioning, participating in the nucleic acid synthesis and energy storage in form of adenosine triphosphate (ATP) (34). Magnesium works as a stabilizer for the nervous system, acts in muscle contraction and it is an alternative to calcium since it is an activator of alkaline phosphatase. Calcium is mainly involved in skeletal growth, 97% is located in bone tissue, and it also works as a co-enzyme in metabolic regulation. It is essential in many processes like the blood clotting cascade, muscle contraction and regulation of sodium ions crossing cell membranes (33,34). Manganese is only required in smaller amounts for proper carbohydrate metabolism and to prevent oxidation of superoxide dismutase enzymes. Additionally, iron is an important element for the formation of haemoglobin in red blood cells. Finally, zinc is known for working as a co-enzyme for about 200 different enzymes present in the immune system, cell growth activities and acid-base regulation (33).

1.1.8. Vitamins

Vitamins are essential nutrients to the human organism. Each of them has specific functions important for normal metabolism, growth and vitality processes. These compounds are found in food, usually in very small amounts. An adequate daily amount of vitamins, as well as minerals, are important for the body to remain healthy, physically and mentally. Vitamin deficiency can lead to serious diseases like obesity, diabetes and heart diseases (21,35).

Vitamins are divided into water soluble (vitamin B12, vitamin C, folic acid, vitamin B3, vitamin B1 and B2) and fat soluble (vitamin K, vitamin E, vitamin D and vitamin A) (35). The most abundant vitamins in peach are vitamin C, E and B-complex vitamins (Table 1) (21). Vitamins E and C have shown preventive effects towards cardiovascular diseases. Vitamin E acts upon low density lipoprotein (LDL) preventing its oxidation and development of atherosclerosis. Vitamin C enhances nitric oxide (NO) production which protects vascular endothelium against free radicals and the ischemic heart from apoptosis (36), and also boosts the immune system, promotes healing and helps prevent cancer (21,36).

1.1.9. Volatile compounds

Volatile compounds are known to have an impact on fruits' flavour and aroma. These characteristics have a dynamic development, they change according to the quantity and quality of the volatile compounds present that vary according to ripening stage and cultivar (16,37). Volatiles can be classified as primary or secondary according to the origin: primary compounds exist in intact fruit tissue, while secondary compounds are only formed after tissue disruption because these are bound to sugars forming glycosides or glucosinolates within the cells (38,39). The secondary volatile compounds are usually in higher number than the primary compounds (free volatiles) and so are a more important source for flavour (38). Flavour volatiles are derived from phytonutrients such as fatty acids, amino acids, carotenoids, phenols and terpenoids (38).

Literature states that more than 100 different compounds have been identified both in peaches and nectarines (16). These are C6 compounds, alcohols, aldehydes, esters, terpenoids, ketones and lactones. The γ- and δ-decalactones, and γ- and δ-dodecalactones have been reported to be of great impact in peach aroma They act in association C_6 aldehydes, alcohols and terpenoids, which are responsible for the spicy, floral and fruity features in the peach (16,37). C_6 compounds are the major volatile compounds in unripe peach and have a “grassy” flavour. The identified C_6 compounds are hexane, hexanal, 2-hexenal, (Z)-3-hexen-1-ol, (E)-2-hexenal, (E)-2-hexen-1-ol and 1-hexanol. As the fruit matures their levels decrease, although, since their origin is from enzyme-catalysed breakdown of fatty acids their levels may still be high in mature peach (16). Esters like ethyl acetate, (Z)-3-hexenyl acetate, hexyl acetate and 2-hexenyl acetate are responsible for the fruity and floral aroma and often represent the major contribution in peaches (16,38).

1.1.10. Carotenoids

Carotenoids are a group of pigments and that are present in all photosynthetic organisms, being responsible for the yellow and red colours both in flowers and fruits, they are the most widespread group of pigments in nature (40). They can be divided into two groups which are oxygenated molecules (lutein and *β*-cryptoxanthin) and unoxygenated carotenes like hydrocarbon carotenoids. Hydrocarbon carotenoids can be either cyclized, like *α*-carotene and *β*-carotene, or linear, like lycopene (41). They have benefits in the field of optics, both lutein and zeaxanthin have the ability to prevent age-related macular degeneration, and they are also involved in reduction of chronic and degenerative diseases including those triggered by reactive oxygen species (ROS) (8,41).

The main carotenes present in peach are *β*-cryptoxanthin and *β*-carotene, their contents ranging from 60 μg/Kg – 360 μg/Kg (peel) and 60 μg/Kg – 160 μg/Kg (flesh), and from 110 μg/Kg – 3790 μg/Kg (peel) and 40 μg/Kg – 1680 μg/Kg (flesh), respectively (11). The carotenoid *α*-carotene can also be found in a limited number of cultivars, in small quantities, e.g. Champagne (10-30 μg/Kg), Rich Lady and September Sun (100-110 μg/Kg) (11). A more recent study confirmed qualitative carotenoid content, finding the main carotenoids to be *β*-cryptoxanthin and *β*-carotene, and total carotenoid values found were of 0.8 – 3.7, 0.0 – 0.1 and 0.1 – 1.9 mg of *β*-carotene per 100 g of tissue for yellow, white and red fleshed peaches, respectively (42).

Table 1. Composition of peach per 100 grams of fresh weight (adapted from USDA http://fnic.nal.usda.gov/food-composition).

Nutrient		Nutrient	
Basic Chemical composition		**Vitamins**	
Water	88.87 g	Vitamin C	6.6 mg
Energy	39 Kcal	Thiamin	0.024 mg
Protein	0.91 g	Riboflavin	0.031 mg
Fat	0.25 g	Niacin	0.806 mg
Carbohydrates (by difference)	9.54 g	Pantothenic acid	0.153 mg
Fibre	1.5 g	Vitamin B-6	0.025 mg
Total Sugars	8.39 g	Folate, total	4 µg
Sucrose	4.76 g	Choline, total	6.1 mg
Glucose	1.95 g	Betaine	0.3 mg
Fructose	1.53 g	Vitamin A, RAE	16 µg
Galactose	0.06 g	Carotene, beta	162 µg
Maltose	0.08 g	Cryptoxanthin, beta	67 µg
Fatty Acids		Vitamin A, IU	326 IU
Fatty Acids (saturated)	0.019 g	Lutein + zeaxanthin	91 µg
Fatty Acids (monounsaturated)	0.067 g	Vitamin E (alpha-tocopherol)	0.73 mg
Fatty Acids (polyunsaturated)	0.086 g	Tocopherol, gamma	0.02 mg
Phytosterols	10 g	Vitamin K (phylloquinone)	2.6 µg
Amino acids		**Minerals**	
Tryptophan	0.010 g	Total ashes, g	0.43 mg
Threonine	0.016 g	Calcium, Ca	6 mg
Isoleucine	0.017 g	Iron, Fe	0.25 mg
Leucine	0.027 g	Magnesium, Mg	9 mg
Lysine	0.030 g	Phosphorus, P	20 mg
Methionine	0.010 g	Potassium, K	190 mg
Cystine	0.012 g	Sodium, Na	0
Phenylalanine	0.019 g	Zinc, Zn	0.17 mg
Tyrosine	0.014 g	Copper, Cu	0.068 mg
Valine	0.022 g	Manganese, Mn	0.061 mg
Arginine	0.018 g	Selenium, Se	0.1 µg
Histidine	0.013 g	Fluoride, F	4 µg
Alanine	0.028 g		
Aspartic acid	0.418 g		
Glutamic acid	0.056 g		
Glycine	0.021 g		
Proline	0.018 g		
Serine	0.032 g		

1.1.11. Phenolic compounds

Phenolic compounds are a class of phytochemicals that result from plant metabolism and are known for their important properties and biological functions like antioxidant activity, antimicrobial activity, pigmentation and protection against UV light. They are the peach's main source of antioxidant activity (43–45). When consumed they offer protection against common chronic diseases such as cardiovascular diseases like atherosclerosis, neurodegenerative diseases like Parkinson's and Alzheimer's diseases and certain types of cancer (43).

The basic structure consists of an aromatic ring with one or more hydroxyl groups. According to their structure they can be divided into two categories: polyphenols and single phenols (46). Single phenols as the name indicates only have one aromatic ring, while the polyphenols have more than one. Polyphenols are classified according the number of aromatic rings and the elements that bind them together. Two main groups are described in the literature, as having different structural characteristics – non-flavonoids, which include stilbenes and phenolic acids; and flavonoids, which include anthocyanins, flavones, isoflavones, flavanones, flavonols and flavan-3-ols (43,47). Additionally, they exist in free, soluble conjugated (glycosides) and insoluble bound forms. The insoluble forms are usually bound to cellular wall of the plants (48).

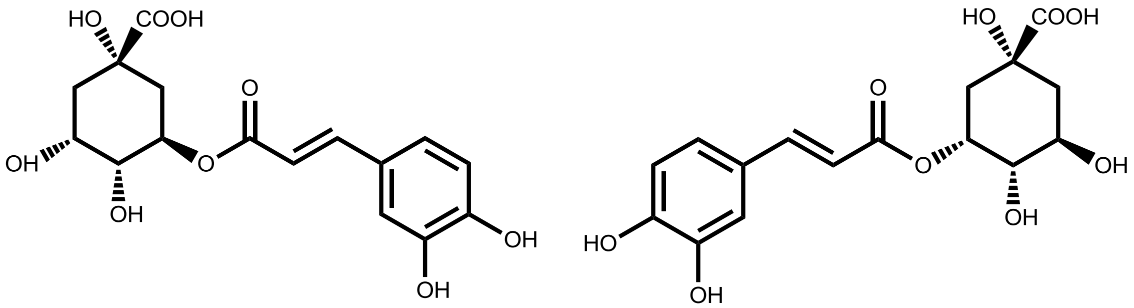
In peaches an inverse proportion between anthocyanin content and other phenolic compounds was observed, as well as the difference in distribution of compounds between peel and flesh. Taking in account the weight percentage peel and flesh represent, the total distribution of the compounds was estimated to be approximately 30% and 70%, respectively (42).

1.1.11.1. Non-flavonoids

These phenolic compounds are single aromatic ring structures known as phenolic acids. These occur in all soluble free, soluble conjugated and insoluble bound forms, unlike flavonoids and tannins which are mainly glycosylated. Their basic structure is usually C6-C1 or C6-C3 (1,2,43,49).

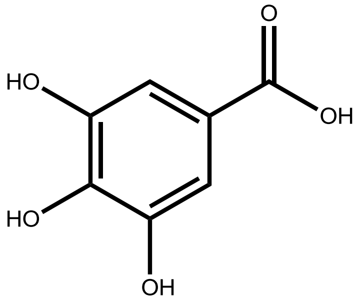
These insoluble forms have a higher anti-oxidant activity compared to the free and conjugated forms, but they only represent 24% of total phenols (50). They are bound to cellulose molecules on cell walls through covalent bonds for example. Since cell walls are difficult to digest the bound phenols are only absorbed in the colon, the only location of the intestine with the appropriate microflora to break down these molecules. These present chemo-protective properties against colon cancer, while the other phenols are absorbed easily and distributed through the body after digestion (50,51).

Phenolic acids can be divided into two subgroups: hydroxybenzoic and hydroxycinnamic acids (46). Hydroxybenzoic acids derive from the benzoic acid, possess 7 carbon atoms and generally have a C6-C1 structure. Hydroxybenzoic acids include *p*-hydroxybenzoic, vanillic, protocatechuic, syringic, salicylic and gallic acids. Hydroxycinnamic acids are also aromatic compounds, but with an additional three carbon side chain making its structure a C6-C3. The most common hydroxycinnamic acids are 5-*O*-caffeoylquinic, 3-*O*-caffeoylquinic, *p*-coumaric, ferulic and sinapic acids (43,51). These acids can be found in free or conjugated forms. The free forms are usually artefacts resulting from chemical or enzymatic hydrolysis, as for the conjugated forms, these are esters of shikimic, tartaric, quinic and other hydroxyacids, also including the sugar by-products (51). The main phenolic acids found in peach are the hydroxycinnamic acid derivatives: chlorogenic acid and its isomer, neochlorogenic acid. Gallic acid can also be found but in lower concentrations compared to the hydroxycinnamates (Figure 3) (6,52).



5-*O*-caffeoylquinic acid

3-*O*-caffeoylquinic acid

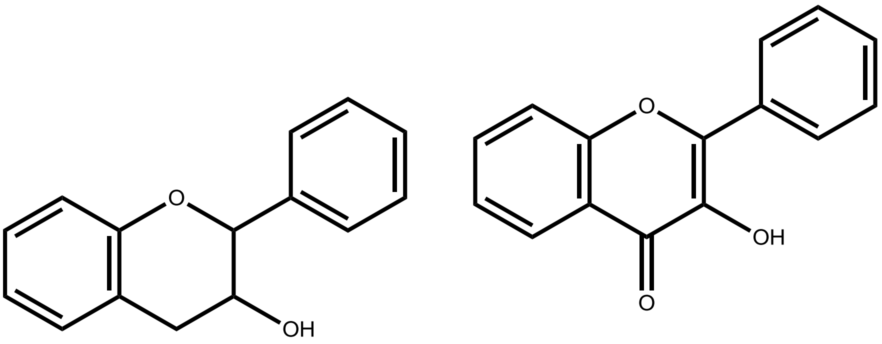


Gallic acid

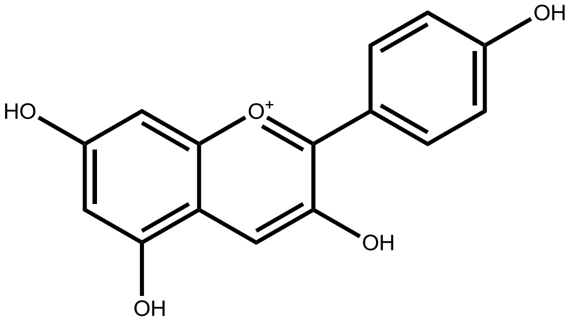
Figure 3. Main phenolic acid content in peach: hydroxycinnamic acids 5-*O*-caffeoylquinic acid and isomer 3-*O*-caffeoylquinic acid.

1.1.11.2. Flavonoids

Flavonoids are low molecular weight molecules, an ensemble of 15 carbons arranged in C6-C3-C6 structure – two aromatic rings (A and B) and a carbon bridge. Usually, this bridge forms a heterocyclic ring known as the C ring, which is condensed with A ring and connected to B ring through C-2 (43,53). Ultimately, flavonoids' structure is based on a flavan nucleus (53). According to different substitutions to the C ring, the flavonoids can be organized in different classes: anthocyanins, flavones, isoflavones, flavanones, flavonols and flavan-3-ols. This ring sometimes has a carbonyl group attached to C-4, forming a *4-oxo-flavonoid* nucleus which is the base structure for flavanols, flavonols, flavanones and flavones. Additionally, substitutions may also occur in A and B rings within the mentioned classes: the flavonoids can be hydroxylated, methylated, acetylated or sulphated (2,53–57). This group of polyphenols is known for being H^+ donors, having metal chelating properties and high redox potential. This offers various protective effects against UV light, pathogens and oxidative stress in cells (43,54). From the different classes only a few of them are actually present in the peach's composition (Figure 4):



Flavan-3-ol **Flavonols**



Anthocyanidin

Figure 4. Flavonoids present in peach.

a. Flavonols are ketone-containing flavonoids with a 3-hydroxyflavone backbone (52). They are the most common subclass of flavonoids, being well distributed in nature, and are also the flavonoid subclass with the highest number of structural variations. The main dietary flavonols are kaempferol, quercetin, isorhamnetin and myricetin, and are typically found as glucosides (1,2,58).
b. Anthocyanins are spread through nature, but are mostly present in flower tissues and fruits like grape skin. They are responsible for the colours red, blue and purple (2). The most predominant anthocyanidins are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin. These compounds are found in plants where they are conjugated with sugars forming anthocyanins (2,47,59). Therefore anthocyanins have an aglycone form called anthocyanidins, although these do not occur in plants as mentioned before (53). The anthocyanin is a glycosylated form. They may also be found conjugated with hydroxycinnamates and organic acids (2).

c. Flavan-3-ols can range from simple monomers such as (+) catechin and its isomer (-)-epicatechin to a hydroxylated form called gallocatechins. These former ones can undergo esterification with gallic acid forming complex structures like polymeric and oligomeric proanthocyanidins, also known as condensed tannins (46). These other structures derive from subtle changes such as a different or additional chiral centre, which have a big impact on the 3D aspect of the molecule, but no impact on its redox properties (2). Flavan-3-ols are the most complex subgroup of flavonoids and have been reported to possess several health benefits such as antioxidant, anti-carcinogenic, cardio-protective, antimicrobial, anti-viral, anti-inflammatory, anti-parasitic and neuro-protective properties (46,52,60).

1.2. Biological activity

1.2.1. Free radicals and oxidative stress

Free radicals are molecules with an odd number of electrons making them highly reactive and transient. Product of natural metabolism or, they can be formed due to ionizing radiation exposure, redox cycling triggered by some drugs, or even xenobiotics capable of forming these odd number molecules *in situ* (61,62). These molecules are toxic to cells, triggering a number of reactions like lipid peroxidation, oxidation of cellular components such as DNA, membrane unsaturated fatty acids and proteins (41,61). This is called oxidative stress, which happens when there’s an imbalance between oxidants and antioxidants (41). This contributes to the aging process and is responsible for many chronic diseases such as cancer, cardiovascular disease, neurodegenerative disorders, atherosclerosis, alcohol induced liver disease and ulcerative colitis (41,61–63).

The cell damage depends on the type of free radical and where it was generated. There are many different types of free radicals like those derived from oxygen – reactive oxygen species (ROS): superoxide ($O_2^{\bullet -}$), hydroxyl (OH^{-}) or peroxyl (ROO^{-}). There are also ROS with an even number of electrons such as hydrogen peroxide (H_2O_2) and lipid peroxide (ROOH), in other words, they are not free radicals as the ones mentioned before, rather non radical derivatives. Reactive nitrogen species (RNS) like nitric oxide ($^{\bullet}NO$) or peroxynitrite anion ($ONOO^{-}$), are also very common given that NO is produced in many cells and tissues from l-arginine by three enzymes called endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) (61,64–66). The inducible type, iNOS, is expressed by inflammatory cells when stimulated by microbe endotoxins or cytokines from tissue injury (66,67).

1.2.1. Antioxidant activity

Organisms have natural defences against these reactive species, enzymatic and non-enzymatic (61,63). Superoxide dismutase (SOD), catalase, glutathione reductase (GR) and glutathione peroxidase (GPx); or even low molecular weight antioxidants like ascorbic acid and *β*-carotene and high-molecular weight antioxidants like ferritin, albumin or ceruloplasmin are able to counteract the harmful effects of free radicals (61,63,68). This protection might be enhanced by a dietary intake of antioxidants, which may play an important role in disease prevention, improving life quality and postponing the onset of degenerative diseases (63).

Antioxidants have been defined as substances that are able to compete with other substrates susceptible to oxidation. They will either delay or inhibit the reaction on these substrates. As mentioned before, these substances can be enzymes, or compounds such as *β*-carotene, ascorbic acid or phenolic compounds (68,69). The antioxidant activity attributed to phenolic compounds its thanks to their ability to perform tasks like free-radical scavenging, donating hydrogen atoms or electrons and chelating metal cations (54). An antioxidant has some specific characteristics that define them as such: reduction potential as electron donors, the ability to delocalize and stabilize the unpaired electron, metal-chelating abilities and reactivity to other antioxidants (69).

The antioxidant properties of phenolic acids are mainly due to the hydroxyl groups and presence of a carbonyl group which will enhance their antioxidant capacity. The relative position of both groups also influences this activity (54). Hydroxybenzoic acids with a single hydroxyl group (monohydroxybenzoic acids) in the *ortho*- or *para*- positions to the carboxyl group exhibit no antioxidant activity, with exception for the 3-hydroxybenzoic acid. The antioxidant ability of phenolic acids increases with the number of hydroxyl groups, making gallic acid a powerful antioxidant since it is a trihydroxybenzoic acid (54). On the other hand, hydroxycinnamic acids have higher antioxidant capacity, probably due to their CH=CH-COOH group, which allows higher ability to donate hydrogen and better radical stabilization than hydroxybenzoic acids (54). Flavonoids, on the other hand, are more complex than phenolic acids due to their flavan based structure. Balasundram *et al.* (2006) has described flavonoids' antioxidant activity to be dependent on various structural variations. The antioxidant activity of flavonoids is mainly due to the number and position of hydroxyl groups, the double bond between C-2 and C-3 and 4-*oxo* group in the C ring, and the catechol group (*ortho*-dihydroxyl) in B ring (54,70,71). The presence of both the 4-oxo group and the C-2 and C-3 double bond on C-ring enhance flavonoids' radical scavenging ability. Besides these features, some flavonoids like kaempferol have an additional hydroxyl group in C-3, further enhancing its radical scavenging abilities (54). The blocking or removal of this hydroxyl decreases antioxidant activity and this blockage may happen through glycosylation, like in quercetin-3-O-rutinoside. Also, if that double bond is removed, the ability of transferring electrons from the B ring to the A ring is blocked, reducing antioxidant activity even further (71). When it comes to the B ring, the presence of hydroxyl groups in positions 3'-, 4'- and 5'- may increase antioxidant activity in some flavonoids (anthocyanindins), but it can also have pro-oxidative effects in others (catechins) (54). On the other hand, the catechol group results in higher antioxidant potential, enhanced lipid peroxidation and is also an important feature in the metal chelation process, acting as a binding site for trace metals such as iron and copper (54,70,71). A study demonstrated the importance of this catechol group with the two hydroxyl groups placed in an *ortho*-position. According to Rice-Evans *et al*. (1996) this position confers the molecule an antioxidant activity of 4.7 Trolox equivalent antioxidant activity (TEAC), like in quercetin. After they were switched to a *meta* position from each other, this activity decreased to a 2.55 (TEAC), close to catechin's activity (72). Corroborating this information, Vizzoto *et al*. (2007) confirmed that there is better correlation between antioxidant capacity and phenolic compound content in comparison to carotenoids and ascorbic acid. This antioxidant potential is, of course, influenced by the phenolic qualitative profile (type of compounds present) and quantitative profile (relative amounts and proportions) of the fruit (42).

The reduction potentials of flavonoid radicals are lower than those of alkyl peroxyl radicals and the superoxide radical, meaning they can inactivate the ROS and thus prevent the consequences of their reactions (72). However, their role as *in vivo* antioxidants is slightly compromised due to metabolism. Both glycoside and aglycone forms may be metabolized in the intestine - flavonoids are degraded to phenolic acids in the colon, and the resulting metabolites might still be further metabolized in the liver (72). These metabolites have a reduced antioxidant activity and reduced effectiveness compared to their aglycone form, and the concentrations of these compounds in organs like the brain are lower than those of smaller molecules like ascorbic acid. Still, the concentrations found are still sufficient to act upon receptors, enzymes and transcription factors. Many protein kinases are influenced by flavonoids acting in many signalling cascades and thus playing a significant role in neurodegenerative diseases (71).

1.3. Peach and health promoting activity

Phenolic compounds present in peach like quercetin, catechins and cyanidin derivatives have been found to play important roles due to their antioxidant, antimicrobial and anti-inflammatory properties (54,58,73,74). Evidence has risen about their preventive effects on multiple chronic and age-related diseases such as diabetes, obesity, hypertension, inflammation, cardiovascular, neurodegenerative and oncologic diseases (1,54,73,75,76).

Several studies have been made with peach and peach extracts to test their effects on the prevention or even as part of treatment for such diseases. As an antidiabetic, peach is a natural α-glucosidase inhibitor. The inhibition of this enzyme is an effective way of reducing postprandial blood glucose sugar levels thus slowing the development of diabetes, and helping insulin-independent diabetics controlling their levels (77). Different extracts prepared from peach have been able to efficiently inhibit protein glycation. Protein glycation is a spontaneous reaction involving sugars, proteins and lipids that leads to chemical modifications which are at the base of diseases like diabetes. Glycation is also implicated in cardiovascular and neurodegenerative diseases, since this reaction leads to production of of ROS and other inflammation enablers (73). The purpose of inflammation is to end infection, repair any existing damage and restore balance of homeostasis. Up-regulated inflammatory responses are linked to the development of chronic diseases such as atherosclerosis, obesity, diabetes, neurodegenerative diseases and sometimes cancer. During inflammation, ROS and NO anions are produced by activated phagocytes and macrophages, respectively. NO reacts with free radical producing peroxynitrite that directly oxidizes low density lipoproteins (LDL), causing irreversible damage to cell membrane. This inflammatory consequence can be diminished by flavonoids' capacity to scavenge free radicals (including NO) preventing them from reaction with NO, thus reducing damage of the cells. Furthermore, flavonoids and other phenolic compounds are able to modulate pro-inflammatory enzymes like phospholipase A2, cyclooxygenase, lipoxygenase and iNOS (66,67). Besides protection against oxidative damage towards lipids and proteins, daily intake of peach peel and fresh pulp derived products also showed important anti-inflammatory capacity, blocking inflammation mediators such as tumor necrosis factor-*α* (TNF-*α*), interleukin IL-1*β* and nuclear factor κB (NF-κB) (41,67,73).

In another instance, both diabetes and inflammation are risk factors for the development of cardiovascular diseases. The incidence of these diseases is influenced by several risk factors such as smoking, diabetes mellitus, hypertension, elevated LDL, low levels of HDL and obesity (78–80). In fact, obesity worsens every other risk factor and it negatively affects cardiac structure and function by increasing blood volume, stroke volume and cardiac output (80). Vascular inflammation is another worsening factor of LDL accumulation and oxidation in the subendothelial space. As a consequence of cardiovascular risk factors, endothelial dysfunction occurs increasing T cell and mast cell binding, leading to an inflammatory process in the arterial wall (79).

Flavonoids prevent LDL oxidation by eliminating ROS, thus preventing/decreasing blood vessel inflammation. This happens by inhibiting the activity of enzymes such as: xanthine oxidase, NADPH oxidase and lipoxygenase. Blood vessel inflammation and platelet aggregation is regulated through a decrease of prostaglandin PGE2, leukotriene B4 and thromboxane A2 due to the inhibitory effects produced in the respective enzymes (76). Besides preventing LDL oxidation, it may increase high-density lipoprotein (HDL) levels and lower the LDL levels in plasma, in a dose dependent manner (1). Angiotensin II is an important vasoactive hormone that has been associated with development of cardiovascular diseases. As it binds to the angiotensin type 1 receptor (AT1 receptor), it activates phospholipase C forming secondary messengers which ultimately leads to an increase in intracellular Ca^{2+}. As shown in Figure 6 Ca^{2+} elevation leads to phosphorylation of myosin light chain (MLC) and activation of nicotinamide adenine dinucleotide phosphatase (NAD(P)H) oxidase leading to vasoconstriction, rise in blood pressure and high production of ROS (81–83). An extract prepared from peach pulp using ethyl acetate was able to inhibit angiotensin II signalling effects. Signal transduction was attenuated through inhibition of Ca^{2+} inflow, inhibition of ROS and blocking of EGFR activation, attenuating cell hypertrophy. The same extract was able to inhibit both EGFR and MYPT1 phosphorylation. This can lead to decreased activation of MLC inducing cellular relaxation (83).

Inflammation is also related to age deterioration of the brain and is associated with the presence of ROS, meaning that the first may be a consequence of the second or vice-versa. The microglial cells activated in inflammation produce high levels of ROS and the induced oxidative stress is linked to the development of Parkinson's and Alzheimer's diseases (84,85). Phenolic compounds are related to neurodegenerative disease prevention, such as Parkinson's and Alzheimer's, namely flavonoids which seem able to cross the blood-brain barrier (76,85). Very few studies have been made in this area involving peach. However, very positive results have been achieved already. Vegetable and fruit extracts, including peach, have shown inhibitory activity (approximately 80% for peach extract) against advanced glycation end-products (AGEs), which are associated with the development of several health disorders like Alzheimer's disease and aging (86). Finally, peach juice was able to successfully inhibit cholinesterases, responsible for the hydrolysis of acetylcholine and thus terminating the transmission of the nerve impulse. The enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are product of two different genes located at chromosomes 7 and 3, respectively. Ache is responsible for 80% of the cholinesterases activity, it terminates the acetylcholine action at the post-synaptic membrane in the neuromuscular junctions. The BChe is only responsible for acetylcholine hydrolysis. Cholinesterase inhibitors are part of Alzheimer's disease treatment, and can also be of use against Parkinson's disease, traumatic brain injury, *myasthenia gravis*, among other severe disorders (87,88). It has been shown that the activity of these two genes is altered in brains with Alzheimer's disease, marked by an increase in BChE activity. Additionally, both enzymes can form *β*-amyloid plaques and neurofibrillary tangles in the brain. In a study performed by Szwajgier and Borowiec a 100% inhibition of both AChE (6.10 ± 0.58 $\mu mol/dm^{-3}$) and BChE (8.57 ± 0.84 $\mu mol/dm^{-3}$) with peach juice (87).

Finally, peach extracts have also shown promising results in the remission of cancer cells, namely breast tumor cells (89,90). In a study performed by Noratto and collaborators using yellow-fleshed peach "Rich Lady", extracts were prepared and tested using xenograft models in female mice. Results were promising, showing inhibition of tumour growth after 12 days of administrating 0,8 – 1,6 mg of chlorogenic acid equivalents/day along with a decreased neoangiogenesis. Also, at a genetic level, the peach extract was able to inhibit the expression of some MMPs genes, which are related to tumour invasion of surrounding tissues, angiogenesis and metastasis. Moreover, quercetin was able to increase inhibitory effect of chemotherapy using cisplatin in a non-toxic dosage and inhibited murine melanoma B16-BL6 cell-line from colonizing lung tissue (90). They concluded that for a human weighing 60 Kg the phenolic intake would be around 370.6 mg/day, and according to their calculations this could be achieved by eating only two to three Rich Lady peaches per day (90).

Another study using two different cancerous breast cell-lines showed that peach extracts had a dose-dependent cytotoxic effect for both estrogen-dependent MCF-7 and estrogen independent MDA-MB-435 lines. The authors used a crude extract, and four fractions prepared from crude extract, designed by F-I (phenolic acids), F-II (monomeric anthocyanins), F-III (flavonols) and F-IV (polymeric anthocyanins). The crude extract, F-I and F-II stood out for reducing cell viability in both lines, F-I being the most effective (89). The MDA-MB-435 growth was completely inhibited by the crude extract and both F-I and F-II using concentrations ranging from 50 to 150 µg/mL. Although, it is important to mention that the crude extracts had a biphasic effect on MCF-7 cells. This cell line required higher concentrations. F-I extract was able to produce a total inhibition of cell growth, but only at a concentration of 150 µg/mL. Low concentrations like crude extract at 50 µg/mL increased the cell line’s viability. It is believed that some phenolic compounds may have a stimulating effect on cancer cells by altering the cell cycle in small doses, while inhibiting their growth in higher doses (89).

Both studies demonstrated that peach phenolics, especially chlorogenic and neochlorogenic acids, have a remissive action in breast tumour cells (89,90). The MDA-MB-435 seems to be evidently susceptible to the treatment, achieved in both studies, but as for the estrogen dependent line, MCF-7, it may require further tests (89).

Concerning antimicrobial activity few reports exist, that seems to be less explored. Two studies showed inhibitory activity towards *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*, and a third one testing peach leaves’ extracts against parasites, also showing good results exhibiting short paralysis and lethal times (74,91,92).

2. Aims of this study

The aim of this study was to determine and evaluate the phenolic profile of six peach cultivars (*Summer Rich*, *Fidelia*, *Royal Glory*, *Royal Magister*, *Royal Lu* and *Sweet Dreams*) collected from Fundão region (Portugal) and evaluate their biological potential. To accomplish this specific work lines were established:

- Characterization of the phenolic profile (non-coloured and anthocyanins) of six peach cultivars by LC/DAD;
- Validation of a LC/DAD Method for routine determination of non-coloured phenolic compounds in peach;
- Validation of a LC/DAD Method for routine determination of anthocyanins in peach.
- Evaluating the antioxidant capacity of peach hydroethanolic extracts against DPPH•, NO and FRAP;
- Evaluating the inhibitory ability of peach hydroethanolic extracts against α-glucosidase enzyme;
- Evaluating *Royal Lu*'s hydroethanolic extract's capacity to prevent ROO• induced oxidative damage in human erythrocytes, concerning to inhibit hemoglobin oxidation and hemolysis.

3. Materials and Methods

3.1. Standards and Reagents

All chemicals used were of analytical grade. The standard compounds were purchased from various suppliers. 5-*O*-caffeoylquinic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, kaempferol-3-*O*-glucoside, quercetin, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, catechin, epicatechin, epigallocatechin gallate, caffeic, gallic and ferulic acids were obtained from Sigma-Aldrich (St. Louis, MO, USA). Cyanidin, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, were from Extrasynthese (Genay, France). 1,1-Diphenyl-2-picrylhydrazyl (DPPH•), *β*-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitrotetrazolium blue chloride (NBT), α-glucosidase from Saccharomyces cerevisiae (type I, lyophilized powder), phosphate-buffered saline (PBS), trypan blue, 2,2′- azobis (2-ethylpropionamidine) and dihydrochloride (AAPH) were purchased from Sigma- Aldrich (St. Louis, MO, USA). N-(1-Naphthyl) ethylenediamine dihydrochloride, sulfanilamide, 4-Nitrophenyl-alpha-D-glucopyranoside and sodium nitroprusside dihydrate (SNP) were obtained from Alfa Aesar (Karlsruhe, Germany). Methanol analytical, methanol HPLC grade and acetonitrile were from Fisher Chemical. Water was deionized using a Milli-Q water purification system (Millipore Ibérica, S.A:U., Madrid).

3.2. Peach samples

The peach samples were supplied by Cerfundão company, being the fruits collected from the region of Fundão (Portugal) from the same orchard. For this study were used 6 cultivars of *Prunus persica* (L.): *Summer Rich, Fidelia, Royal Glory, Royal Magister, Royal Lu* and *Sweet Dreams*. Nine fruits per cultivar were randomly selected and used for the chemical and biological analysis. They were manually harvested at the same stage of maturation (July 2015), by incising the stalk of the fruit from the main branch. The samples were transported to the laboratory of Faculty of Health Sciences (Covilhã), cut in half to remove the pit and, then were frozen with liquid nitrogen, lyophilized (SCANVAC CoolSafe™, Frilabo, Portugal), and powdered (mean particle size lower than 910 µm), being divided into three aliquots, extracted, and analyzed separately for chemical composition and biological activity.

3.3. Extraction

For determination of the non-coloured and coloured (anthocyanins) phenolic compounds were extracted according to the method described by Silva and Queiroz (2016) (93). 1 g of dried material was weighed for extraction with 20 mL of ethanol:water (7:3), subjected to a 30 min stirring and 15 min centrifugation at 2900 RCFs. The supernatant was separated and evaporated under reduced pressure. The resulting extract was then dissolved in 50 mL deionised water. The phenolic compound fractions were separated in a Sep-Pak C18 column. The preconditioning of the solid-phase extraction cartridge was performed with 20 mL of ethyl acetate, 20 mL of methanol and 20 mL of 0.01 mol/L HCl. After the addition and passage of the sample, the column was washed with 3 mL of 0.01 mol/L HCl. Fraction I, designed by non-coloured phenolics was eluted with 20 mL of ethyl acetate; Fraction II, designed by anthocyanins was eluted with 40 mL of ethanol containing 0.1% HCl. Both fractions were evaporated under reduced pressure. Finally, the coloured phenolics (fraction II) were re-dissolved in 2 mL of acidified water (pH 3.0), and the non-coloured phenolics (fraction I) re-dissolved in 2 mL of methanol and filtered using a membrane filtered (0.45 µm). The extract used for the biological assays consists at the junction of fraction I and II and evaporated under reduced pressure, with an average yield of 5.0 ± 1.2%.

3.4. Phenolic profile analysis

The extracts were analysed on an analytical LC system (1260) from Agilent Technologies (Waldbronn, Germany) with an auto-sampler and coupled to diode array detector (Agilent 1260 Infinity series), using a Nucleosil® 100-5 C_{18} column (25.0 cm ×0.46 cm; 5 µm particle size waters, (Macherey-Nagel, Düren, Germany), based on the conditions previously described by Silva and Queiroz (93). The chromatographic system was controlled by Chemstation software supplied by Agilent Technologies (Waldbronn, Germany).

3.4.1. Coloured phenolics (anthocyanins)

The conditions used were previously reported by Silva and Queiroz (2016). The mobile phase consisted of water/formic acid/acetonitrile (87:10:3, v/v/v; eluent A) and water/formic acid/acetonitrile (40:10:50, v/v/v; eluent B) using a gradient program as follows: from 10% to 25% B (10 min), from 25% to 31% B (5 min), from 31% to 40% (5 min), from 40% to 50% B (10 min), from 50% to 100% B (10 min), from 100% to 10% B (5 min). Total run time was 50 min. Flow rate was 0.8 mL/min. The injection volume was 20 µL. Detection was achieved with a diode-array detector (DAD-Agilent, 1260 series). The compounds in each sample were identified by comparing their retention times and UV-Vis spectra in the 200–600 nm range with the library of spectra previously compiled by the authors. Anthocyanin quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. Anthocyanins quantification was achieved using a calibration plot of external standard at 500 nm. The compound unknown 1 was quantified as cyanidin-3-*O*-glucoside.

3.4.2. Non-coloured phenolics

The conditions used were previously reported by Silva and Queiroz (2016).The mobile phase used is composed by 2% (v/v) acetic acid in water (eluent A) and 0.5% (v/v) acetic acid in water and acetonitrile (50:50, v/v, eluent B). The solvent system starting with 10% of B, and installing a gradient to obtain 24% B at 20 min, 30% B at 40 min, 55% B at 60 min, 70% B 65 min, 80% B at 70 min), 100% B at 75 min, and maintain 100% B isocratic during 5 min (80 min). A solvent flow rate was 1.0 mL/min. The injection volume was 20 µL. Detection was achieved with a diode-array detector (DAD-Agilent, 1260 series). Spectral data from all peaks were accumulated in the range of 200–400 nm and chromatograms were recorded at 280 (flavan-3-ols and hydroxybenzoic acids), 320 (hydroxycinnamic acids) and 350 nm (flavonols). Peak purity was checked by the software contrast facilities. Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. The hydroxybenzoic acid derivative was quantified as p-hydroxybenzoic acid; 3-*O*-caffeolquinic acid, caffeic acid and hydroxycinnamic derivatives 1, 2 and 3 were quantified as 5-*O*-caffeolquinic acid; and finally catechin derivatives 1 and 2 were quantified as catechin.

3.5. Antioxidant activity

The antioxidant potential of peach extracts was tested against FRAP, DPPH and NO radicals.

3.5.1. Ferric reducing antioxidant power (FRAP)

FRAP assay was performed according to the colorimetric method described by Benzie and Strain (1996). The method measures the compounds’ ability to reduce ferric iron (Fe^{3+}) through reduction of the Fe^{3+} and 2,4,6-tripyridyl-s-triazine (Fe^{3+}-TPTZ)complex to ferrous form (Fe^{2+}-TPTZ) at low pH. The working FRAP reagent was prepared with 2.5 mL of acetate buffer (300nM, pH 3.6), 0,25 mL of TPTZ (10mM in 40 mM HCl) and 0,25 mL of FeCl3•H2O (20 mM).For each peach extract the final concentration used was 2,5 mg/mL. The antioxidant activity of the samples was determined against standards of L-ascorbic acid by monitoring the changes in absorbance at 593nm at T0’ and T40’ due to the reduction of Fe^{3+}-TPTZ complex to a coloured Fe^{2+}-TPTZ complex induced by the samples (63,94). Absorbance was corrected using a blank of H_2O instead of the sample and L-ascorbic acid was used as standard.

$$FRAP\ value_t = \frac{Abs_{sample\ 40'} - Abs_{sample\ 0'}}{Abs_{standard} - (Abs_{standard} - Abs_{blank})} \times 2$$

3.5.2. DPPH● assay

Five dilutions were prepared from each extract. From each different concentration 25 µL were added to the 96 well microplate. In the microplate each well contained 25 µL of extract and 200 µL of 150 mM methanolic $DPPH^{\bullet}$. The plate was incubated in a dark during 30 min at room temperature and absorbance was determined at 515 nm (95,96). Ascorbic acid was used as positive control. Three experiments were performed in triplicate.

$$Inhibition\ of\ DPPH^{\bullet}(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}}$$

3.5.3. Nitric oxide assay

For each extract, a dilution series (five different concentrations) was prepared with KH_2PO_4 buffer (100 mM, pH 7.4) in a 96-well plate and the antiradical activity was determined spectrophotometrically in a 96-well plate reader. The reaction mixtures in each well consisted of 100 µL of each dilution and 100 µL of SNP (20 mM). The plates were incubated at room temperature for 60 min in exposure to light. After the incubation 100 µL of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 2% H_3PO_4) was added to the mix, followed by a10 min incubation period. Absorbance of the chromophore formed during the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine was determined at 560 nm (95,96). Ascorbic acid was used as a positive control. Three experiments were performed in triplicate.

$$Inhibition\ of\ {}^{\bullet}NO(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

3.6. α-glucosidase inhibitory activity

Spectrophotometric determinations were based on Ellman’s method, as previously described by Silva *et al.* (95). Dilutions were prepared for each extract (six different concentrations) using KH_2PO_4 buffer (10 mM, pH 7). Each well contained 100 µL of 4-nitrophenyl α-D-glucopyranoside (2.5 mM), 150 µL of KH_2PO_4 buffer (10 mM, pH 7), and 50 µL of the respective dilutions. The absorbance was read at 405 nm. After this step, 25 µL of α-glucosidase (0.28 U/mL) was added and the absorbance was read after 10 minutes of incubation at 37ºC. The rate of the reaction before adding the enzyme was subtracted from that obtained after adding the enzyme in order to correct eventual spontaneous hydrolysis of substrate (96). Acarbose was used as a positive control. Three experiments were performed in triplicate.

$$Inhibition\ of\ \alpha - Glucosidase\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

3.7. In vitro $ROO^{\bullet}$-induced oxidative damage in human erythrocytes

For this assay only one cultivar was tested. *Royal Lu* was chosen due to its good performance in the previous antioxidant assays. The extract was dissolved in PBS and six different concentrations were prepared. All readings were performed in a microplate reader with thermostat. Four experiments were performed (n=4) in duplicate and the IC_{50} values were calculated using the haemolysis inhibition curves on GraphPad Prism software.

3.7.1. Erythrocyte isolation

Venous human blood was collected, by antecubital venepuncture, into tubes containing K_3EDTA and the erythrocytes were isolated based on the procedure described by Chisté *et al.*

and Carvalho *et al* (97,98). Approximately 4 mL of the blood samples were transferred to sterile 15 mL conical centrifuge tubes containing phosphate buffer saline (PBS) at pH 7.4 and centrifuged at 1500xg for 10 min at 4 °C. After centrifugation, erythrocytes were separated from plasma and buffy coat, followed by a washing step. Erythrocytes were washed with 6 mL of PBS followed by centrifugation at 1500xg at 4 °C for 5 min. The procedure was repeated 3 times and erythrocytes were re-suspended using the same buffer. The number of cells (cells/mL) and viability (always above 98%) were obtained by the Trypan blue exclusion method. The suspensions of isolated erythrocytes were kept on ice until use.

3.7.2. Inhibition of hemolysis

The prevention of $ROO^•$-induced hemolysis of human erythrocytes was based on the procedure described by Chisté *et al.* (97). To induce free radical chain oxidation in erythrocytes, $ROO^•$ were generated by thermal decomposition of AAPH (dissolved in PBS; final concentration 17 mM) at 37ºC. The inhibition of haemolysis by the peach extracts was determined by monitoring the release of haemoglobin after membrane disruption caused by the AAPH-induced haemolysis. Five dilutions of our extracts were prepared with PBS. The reaction mixtures were placed in a 48-well plate and consisted of 200 µL of the erythrocyte suspension (17×10^6 cells/mL) and 100 µL of the sample dilution or PBS (blank and control). These were first incubated in a water bath at 37 °C, during 30 min, with slow agitation (50 rpm), followed by the addition of 200 µL of AAPH in the media, except in the blank experiment. The reaction mixture was incubated at 37 ºC for 3 h with slow agitation. After the incubation time in the presence of AAPH, the reaction volume (500 µL) was transferred to 1.5 mL Eppendorf tubes and centrifuged at 1500xg for 5 min at 4 °C. The supernatant (300 µL) was placed in a 96-well plate and the absorbance was read at 540 nm. The results were expressed as IC_{50} values (µg/mL).

$$\text{Inhibition of haemolysis (\%)} = 100 - \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}}\right) \times 100$$

3.7.3. Inhibition of hemoglobin oxidation

The venous blood erythrocyte suspension preparation was based on the same method mentioned in the above paragraph and the erythrocyte suspension was prepared with a density of 500×10^6 cells/mL (97,98).

The induction of the haemoglobin oxidation was based on the procedure described by Chisté *et al.* (97). The $ROO^•$ radicals generated by the thermal decomposition of AAPH (dissolved in PBS; final concentration 50 mM) at 37ºC react with oxyhemoglobin forming methaemoglobin. The inhibition of oxyhaemoglobin oxidation by the extracts was determined by monitoring formation of methaemoglobin which can be accompanied spectrophotometrically at 630 nm.

Five dilutions of our extracts were prepared with PBS. Reaction mixtures were prepared with 200 µL of the erythrocyte suspension and 100 µL of the sample dilution or PBS (blank and control) in a 48-well plate, followed by incubation in water bath at 37 °C, during 30 min, with slow agitation (50 rpm). After the incubation 200 µL of AAPH were added to the media (except in the blank) followed by a second incubation in the water bath at 37 ºC for 4 h with slow agitation. After the incubation the reaction volume (500 µL) was transferred to 1.5 mL Eppendorf tubes and centrifuged at 1500 g for 5 min at 4 °C. The supernatant (300 µL) was placed in a 96-well plate and the absorbance was read at 630 nm. The results were expressed as IC_{50} values (µg/mL).

$$\text{Inhibition of haemoglobin oxidation (\%)} = 100 - \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}}\right) \times 100$$

3.8. Statistical Analysis

All data were recorded as mean ± standard deviation of triplicate determinations. Mean values were compared using one-way analysis of variance (one-way ANOVA) (Graph Pad Prism Version 6.01, GraphPad Software, Inc., San Diego, CA). Differences were considered significant for $P < 0.05$. Significant results ($P<0.05$) are indicated as: a – *vs Summer Rich*; b – *vs Fidelia*; c – *vs Royal Glory*; d – *vs Royal Magister*; e – *vs Royal Lu*.

4. Results

4.1. Phenolic profile analysis

4.1.1. Anthocyanins

The LC-DAD analysis of the coloured fraction extracted from the peaches provided the detection of three anthocyanins: unknown (**1**), cyanidin-3-*O*-glucoside (**2**) and cyanidin-3-*O*-rutinoside (**3**) (Table 2 and Figure 5). All compounds were previously described in peaches, except unknown 1 despite not to be identified was herein reported for the first time (8,13,18,99,100).

The calibration curves used to quantify each compound were assessed using various standard solutions at seven different concentrations. All of them showed good linearity, with $R^2 \geq 0.9991$, limits of detection ($LOD = 3S_0/b$) and quantification ($LOQ = 10S_0/b$) (where S_0 is the standard deviation of the signal-to-noise ratio and b is the slope of the calibration plot) ranged from 0.21 to 0.63 ng/mL and 0.63 to 1.91 ng/mL, respectively (Table 3). We can say that the method is sensible being in agreement with previous works (101–103).

The method’s repeatability was evaluated by analysing the same peach sample (Royal Lu) five times in the same day (intraday precision). Repeatability (intraday precision) showed good results with coefficient values inferior to 3% (Table 2). Interday precision was determined by analysing the same peach sample (*Royal Lu*) in 5 different days. Coefficient values were all inferior to 6% (Table 1), indicating that the method is precise enough for the studied matrix (101–104).

Recovery of anthocyanins was evaluated with an aliquot of cyanidin-3-*O*-glucoside standard solution that was quantified using the same method. The recovery was of 88±4.3%, showing a good pattern according to previous studies (105–108).

Table 2. Anthocyanins content of *Prunus persica* from Fundão region (μg/g).

Phenolic compound	Regression equations	R^2	LOD (ng/mL)	LOQ (ng/mL)	Repeatability (CV%)	Interday precision (CV%)	*Summer Rich*	*Fidelia*	*Royal Glory*	*Royal Magister*	*Royal Lu*	*Sweet Dreams*
Unknown	Y=113.64X+35.697	0.9992	0.21	0.63	2.03	4.64	0.03 ± 0.0	nq	nq	nq	0.47 ± 0.0^{a}	nq
Cyanidin-3-O-glucoside	Y=113.64X+35.697	0.9992	0.21	0.63	1.52	5.06	20.4 ± 0.2	1.5 ± 0.0 ^{a}	49.5 ± 3.2 ^{ab}	135.2 ± 0.2 ^{abc}	46.5 ± 0.6 ^{abd}	42.3 ± 0.1 ^{abcde}
Cyanidin-3-O-rutinoside	Y=37.773X+23.532	0.9991	0.63	1.91	1.04	5.24	3.6 ± 0.6	1.1 ± 0.1^{a}	4.7 ± 0.3 ^{ab}	18.2 ± 0.1 ^{abc}	7.3 ± 0.1 ^{abcd}	8.4 ± 0.0 ^{abcde}
						Σ	**24.03**	**2.6**	**54.2**	**153.4**	**54.3**	**50.7**

The values are expressed as mean ± standard deviation of three determinations; ∑ sum of the determined compounds; nd: not detected.

Despite the differences observed in the amounts of each anthocyanin, all the analysed peach samples exhibited a similar profile, being that Unknown anthocyanin was only detected and quantified in *Summer Rich* and *Royal Lu*. The total amounts per cultivar varied from 2.8 µg/g (*Fidelia*) to 153.5 µg/g (*Royal Magister*) (Table 2). These results are supported by another study which confirmed that light flesh peaches have lower anthocyanin levels, being that *Fidelia* is a white flesh cultivar (42). As it was previously described cyanidin-3-*O*-glucoside is present in higher amounts, with contents ranging from 1.6 ± 0.1 to 135.2 ± 0.2 µg/g of dried fruit, followed by cyaniding-3-*O*-rutinoside varying between 1.2 ± 0.1 to 18.3 ± 0.1 µg/g of dried fruit (Table 1).

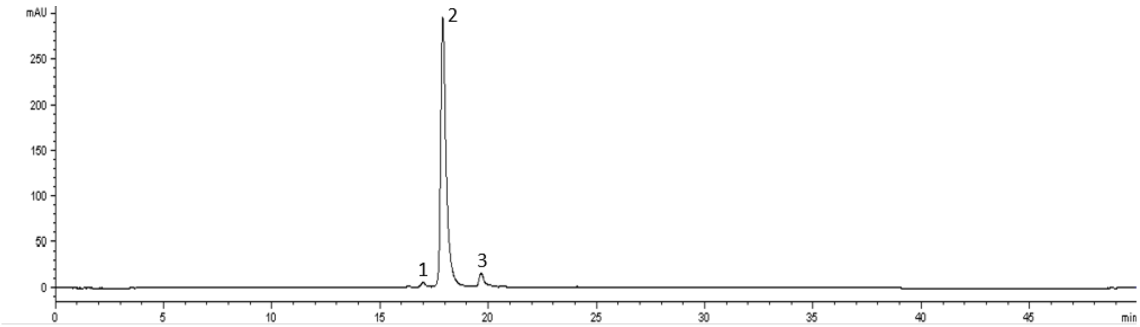


Figure 5. LC-DAD anthocyanins profile of Royal Lu peach. Detection at 500 nm. Peaks: (1) Unknown, (2) cyanidin-3-O-glucoside, (3) cyanidin-3-O-rutinoside.

Anthocyanins are spread through nature, they are important pigments present in flower and fruit tissues like grape skin. They are the glycosylated form of anthocyanidins and they may also be found conjugated with hydroxycinnamates and organic acids (2,109). Their antioxidant properties make them important at preventing chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases (109).

4.1.2. Non-coloured phenolic compounds

The analysis by LC/DAD of peach samples allowed the identification and quantification of fourteen non-coloured phenolics, which included: one hydroxybenzoic acid (**1**), eight hydroxycinnamic acids (**2**, **4-8** and **10**), three flavan-3-ols (**3**, **9** and **11**) and three flavonols (**12-14**) (Table 3, Figure 6). All of these compounds were previously described in peaches, even though there are evident qualitative and quantitative differences (Table 2) (6,12,13,52,99). The hydroxybenzoic derivative was not identified in *Fidelia* nor *Sweet Dreams*, 3-*O*-caffeolquinic acid and catechin derivative 2 were not identified in *Royal Lu*, catechin was not identified in *Summer Rich*, hydroxycinnamic acid derivative 1 was not identified in *Royal Glory* nor *Royal Lu*, hydroxycinnamic acid derivative 2, ferulic acid and quercetin were only identified in Royal Lu, caffeic acid was only detected in Royal Lu and

Sweet Dreams, hydroxycinnamic acid derivative 3 was only identified in *Royal Magister* and catechin derivative 2 was not detected in *Royal Lu* (Table 3).

The calibration curves used to quantify each compound were assessed using various standard solutions at seven different concentrations. All of them showed good linearity, with $R^2 \geq 0.9991$, LOD and LOQ ranged from 0.15 to 1.18 ng/mL and 0.46 to 3.59 ng/mL, respectively (Table 3). We can say that the method is sensible, being in accordance with previously reported (110–112).

Repeatability values were inferior to 12%, they ranging from 0.18 % to 11.41% (Table 3). Concerning interday precision, the coefficient values were all inferior to 15%, ranging from 1.13% to 14.76% indicating that the method is precise enough for the studied matrix (Table 3) (105,107,108,110–112).

Recovery was evaluated using 5-*O*-caffeoylquinic acid and rutin standard solution. They were treated and quantified as samples, being obtained 83±1.8% and 88±0.8% for recovery, respectively (Table 3). According to previous studies the values obtained the method shows good recovery pattern (105–108).

Table 3 - Non-coloured phenolic content of *Prunus persica* from Fundão region (µg/g).

	Phenolic acid	Regression equations	R^2	LOD (ng/mL)	LOQ (ng/mL)	Intraday	Interday precision (CV%)	*Summer Rich*	*Fidelia*	*Royal Glory*	*Royal Magister*	*Royal Lu*	*Sweet Dreams*
1	Hydroxybenzoic acid derivative	y = 20.156x + 47.964	0.9953	1.18	3.59	0.18	2.63	287.8 ± 1.9	nq	10.4 ± 0.1 ^{a}	47.9 ± 4.3 ^{ac}	1064.9 ± 1.9 ^{acd}	nq
2	3-*O*-caffeoylquinic acid	y = 57.751x + 74.075	0.9997	0.41	1.25			30.9 ± 1.7	88.7 ± 0.5 ^{a}	33.2 ± 0.7 ^{b}	41.6 ± 0.1 ^{abc}	nd	29.03 ± 0.1 ^{bcd,}
3	Catechin	y = 21.724x + 16.743	0.9983	1.09	3.33	11.41	8.69	nq	3.3 ± 0.0	4.5 ± 0.1	5.9 ± 0.6	20.5 ± 2.3 ^{bcd}	46.5 ± 0.2 ^{bcde}
4	Hydroxycinnamic acid derivative 1	y = 57.751x + 74.075	0.9997	0.41	1.25		14.64	4.6 ± 0.1	7.9 ± 0.0 ^{a}	nq	1.9 ± 0.1 ^{ab}	nq	3.9 ± 0.1 ^{abd}
5	Hydroxycinnamic acid derivative 2	y = 57.751x + 74.075	0.9997	0.41	1.25	1.39	6.77	nq	nq	nq	nq	23.05 ± 0.1	nq
6	5-*O*-caffeoylquinic acid	y = 57.751x + 74.075	0.9997	0.41	1.25	10.72	14.76	59.6 ± 0.2	70.3 ± 4.3 ^{a}	49.5 ± 2.9 ^{ab}	49.8 ± 0.4 ^{ab}	0.8 ± 0.0 ^{abcd,}	81.1 ± 0.1 ^{abcde}
7	Caffeic acid	y = 136.66x + 18.19	0.9999	0.17	0.52	10.92		nq	nq	nq	nq	0.5 ± 0.1	2.8 ± 0.0
8	Hydroxycinnamic acid derivative 3	y = 57.751x + 74.075	0.9997	0.41	1.25			nq	nq	nq	0.7 ± 0.0	nq	nq
9	Catechin derivative 1	y = 21.724x + 16.743	0.9983	1.09	3.33	0.22	1.97	62.4 ± 0.2	25.7 ± 0.0 ^{a}	78.2 ± 0.2 ^{ab}	51.5 ± 0.1 ^{abc}	51.5 ± 0.1 ^{abc}	26.1 ± 1.3 ^{acde}
10	Ferulic acid	y = 136.37x + 154.91	0.9996	0.15	0.46	0.27	1.77	nq	nq	nq	nq	117.7 ± 0.3	nq
11	Catechin derivative 2	y = 21.724x + 16.743	0.9983	1.09	3.33			26.2 ± 1.1	11.01 ± 0.4 ^{a}	32.4 ± 0.2 ^{ab}	34.1 ± 0.1 ^{ab}	nq	23.4 ± 0.1 ^{abcd}
12	**Quercetin-3-*O*-rutinoside**	y = 38.293x - 20.983	0.9999	0.62	1.89	9.77	1.13	4.5 ± 0.5	9.7 ± 0.3 ^{a}	13.9 ± 0.1 $^{a, b}$	18.2 ± 0.1 ^{abc}	5.9 ± 0.3 ^{abcd}	10.8 ± 0.1^{abcde}
13	**Quercetin-3-*O*-glucoside**	y = 59.442x + 8.1806	0.9998	0.59	1.80	11.17	2.95	1.8 ± 0.4	4.4 ± 0.1 ^{a}	9.9 ± 0.0 ^{ab}	12.6 ± 0.1 ^{abc}	0.8 ± 0.2 ^{abcd}	6.5 ± 0.0 ^{abcde}
14	Quercetin	y = 29.006x + 26.772	0.9997	0.44	1.35	10.16	3.98	nq	nq	nq	nq	2.2 ± 0.2	nq
							Σ	**477**	**221.01**	**232**	**264.2**	**1287.8**	**230.1**

The values are expressed as mean ± standard deviation of three determinations; Σ sum of the determined compounds; nd: not detected.

The total amounts of non-coloured phenolics ranged from 221.2 µg/g to 1288.1 µg/g of lyophilized fruit, being *Royal Lu* the richest one and *Summer Rich* the cultivar with lowest contents (Table 2). In previous studies cinnamic acids have been reported to be the main phenolic compounds in peach, and sometimes flavan-3-ols as well, all changing and depending according to the cultivar. Flavonols normally represent the smallest percentage of phenolic compounds in peach (52,99,100). All cultivars in the study seem to follow the same general pattern described before, with the exception of *Royal Lu* which is exceptionally rich in a benzoic acid derivative.

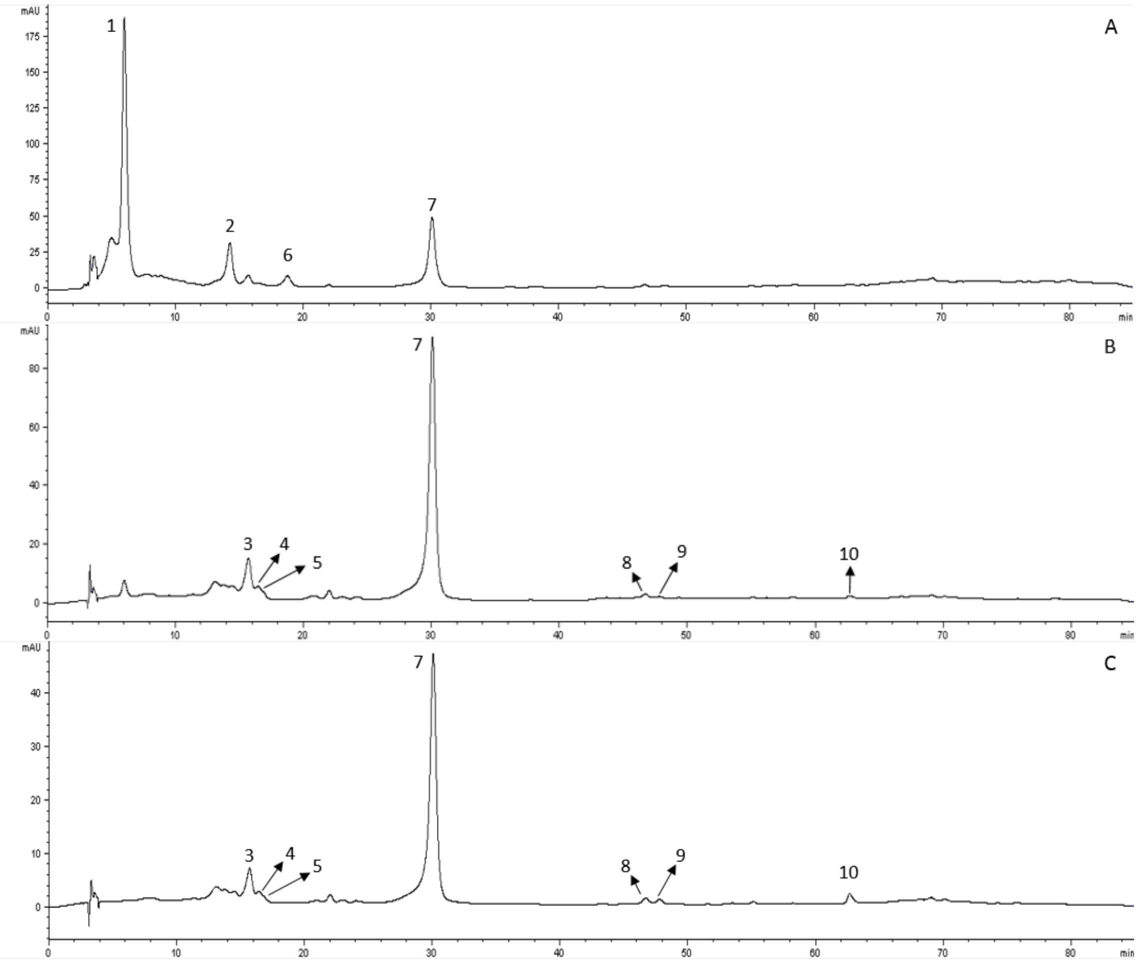


Figure 6. LC-DAD non-coloured phenolics profile of *Royal Lu* peach cultivar from Fundão. A Detection at 280 nm; B: Detection at 320 nm; C: Detection at 350 nm.

Phenolic acids (C_6-C_1) are non-flavonoids which are significantly present in human diet. The most common forms are hydroxycinnamic acids like the ones identified by this method: 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, ferulic acid, caffeic acid and other hydroxycinnamic acid derivatives. They are bound to cellulose molecules from cell walls through covalent bonds (2,50). Both *Royal Lu* and *Summer Rich* presented high contents of hydroxybenzoic acid derivative, 88.7% and 60.2% respectively (Table 3). Benzoic acids are not as common, but gallic acid (most abundant phenolic acid in nature) is a trihydroxybenzoic acid making it an important antioxidant (1,54,72). Some cultivars are not particularly rich in hydroxycinnamic acid derivatives, but both hydroxycinnamic acids 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid were detected, representing 6.5% (Summer Rich) to 40.1% of total non-coloured phenolics (*Fidelia*) and 0.06% (*Royal Lu*) to 35% of total non-coloured phenolics (*Sweet Dreams*), respectively. Ferulic acid was only detected in *Royal Lu*, representing 9.1% of non-coloured phenolic content.

Flavonoids are low molecular weight molecules consisting of 15 carbons arranged in C6-C3-C6 manner – two aromatic rings (A and B) and a carbon bridge which forms a heterocyclic ring designated as the C ring (43). According to different substitutions flavonoids can be organized in different classes: anthocyanins, flavones, isoflavones, flavanones, flavonols and flavan-3-ols (2,43). The compounds that were detected belong to 2 different classes: flavonols and flavan-3-ols. Flavonols are the most common of the flavonoids, being well distributed in nature, and are also the flavonoid subclass with a high number of structural variations (1,2). However flavonols represent the lowest percentage of phenolic compounds detected in the studied cultivars with amounts varying from 0.5% (*Royal Lu*) to 6.9% (*Royal Magister*) and 0.1% (*Royal Lu*) to 4.8% (*Royal Magister*) for quercetin-3-*O*-rutinoside and quercetin-3-*O*-glucoside, respectively. Quercetin was only found in *Royal Lu* representing only 0.2% of its phenolic compound constitution. As for flavan-3-ols, they are well represented in practically every cultivar, varying from 5.6% in *Royal Lu* and 49.6% in *Royal Glory*. This class of flavonoids comprises the most complex set of structures within the flavonoids' group, ranging from simple monomers such as catechin and its isomer epicatechin to hydroxylated forms called gallocatechins. These former ones can undergo esterification with gallic acid forming complex structures like polymeric and oligomeric proanthocyanidins, also known as condensed tannins (2).

4.2. Antioxidant activity

Phenolic compounds are known to possess certain characteristics that have attributed them the designation of antioxidants, characteristics such as reduction potential as electron donors, the ability to delocalize and stabilize the unpaired electrons, metal-chelating abilities and reactivity towards antioxidants, being attributed the designation of antioxidants. They compete with substrates which are also prone to oxidation, thus inhibiting or delaying these reactions between reactive species/free radicals and the substrates (54,69,113). Even though ROS and RNS are involved in several biological processes, overproduction can lead to cell damage and development of diseases. The dietary intake of these antioxidants works as a boost to the organisms' already existing defences against reactive species and free radicals playing an important role in prevention of diseases (61,63,113).

During the development of this work antioxidant activity of *P. persica* was assessed against FRAP, DPPH• and $NO^{\bullet -}$. The scavenging activity against FRAP has been reported before for *P. persica* methanolic extracts as well as the radical scavenging activity against DPPH•

(10,11,74,91,100,114). As far as we know, the radical scavenging activity against $^{\bullet}NO$ was herein reported for the first time in peaches produced in Portugal.

4.2.1. Ferric Reducing Antioxidant Power (FRAP)

FRAP measures antioxidant effect as reducing ability or, in other words, the ability of any antioxidant substance to donate electrons. This is achieved by measuring the change in absorption at 593 nm of the Fe^{3+}-TPTZ complex to coloured ferrous complex Fe^{2+}-TPTZ (63,114). The FRAP assay was selected because is simple to perform to measure the antioxidant activity of fruits, wines, animal tissues, pure compounds among others. The FRAP value of dried extract revealed that *Royal Lu* (13.9 ± 1.2 μM Fe^{2+}) and *Fidelia* (13.8± 1.8 μM Fe^{2+}) were the most active, followed by *Royal Glory* (12.8 ± 4.1 μM Fe^{2+}), *Sweet Dreams* (11.1 ± 0.3 μM Fe^{2+}), *Summer Rich* (7.7 ± 1.9 μM Fe^{2+}) and *Royal Magister* (5.9 ± 1.0 μM Fe^{2+})(Figure 7).

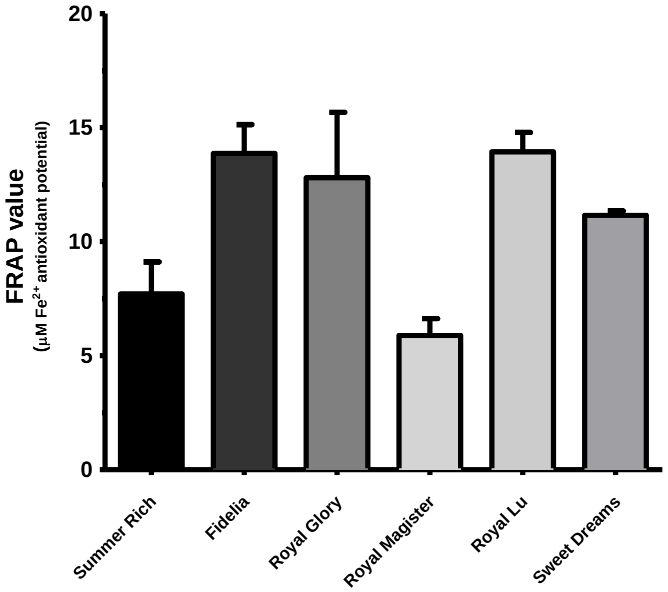


Figure 7. Ferric reducing antioxidant power (FRAP) of peach extracts.

Both *Fidelia* and *Royal Lu* seem to be very rich in phenolic acids. There have been previous reports about phenolic compounds metal chelating properties, namely catechin, but neither *Fidelia* nor *Royal Lu* seem to be particularly rich in this flavan-3-ol, although they do possess two catechin derivatives. Catechins, in its whole, have been labelled as powerful radical scavengers and metal-chelators due to their catechol group in the B ring, despite having a saturated C ring which prevents electron delocalization (72,115). *Fidelia*'s and *Royal Lu*'s flavan-3-ol contents (5.6% and 18.1%, respectively) together with their phenolic acid content (40.1% and 97.9%, respectively) may enhance activity since the phenolic acids can compensate the lack of delocalization and stabilize the resulting aryloxyl radicals through electron donation (72). Still, the differences found in each cultivar's activities lead us to believe that there are other phytochemicals besides the phenolic compound contents that influence the peaches' activity. Taking *Royal Magister* for example, its phenolic acids content consists of 53.6% and flavan-3-ols represent 34.6% of the total contents of non-coloured phenolic compounds, and additionally it is also the cultivar with highest anthocyanin content (153.5 µg/g). However, as it is demonstrated in Figure 7, its ferric reducing activity is the lowest of the sample group. Few studies have explored antioxidant capacity by FRAP assay in peach. In a work performed by Gil and collaborators determined the FRAP of five white flesh cultivars and five yellow flesh cultivars, the obtained results revealed a presence of a clear difference between the two groups: white flesh peaches showed higher reducing activity then yellow fleshed ones. Analysing the FRAP results of nectarines, the same pattern between white and yellow flesh nectarines was observed with FRAP values. Additionally, the authors proved that the plums had a much higher activity than peaches. In this study it is also possible to see that the FRAP is closely related to the total phytochemicals in fruit, given that the plums had a slightly better activity probably due to their higher contents in carotenoids (11). Comparing with other existent data, the activity obtained in this work for the peach extracts was significantly low compared with blackberry extracts, the FRAP activity ranged from 190.8 ± 0.8 µM Fe^{2+} to 191.5 ± 0.4 µM Fe^{2+} for 1 gram of extract. Authors associated this activity to the anthocyanin content present in these berries (116). Another study showed ferric reducing activity for hawthorn leaves. The dried extracts have proven to have reducing activity as well with a FRAP value of 79.2 µM Fe^{2+}(117). Given this information it is important to take in account the phytochemicals that weren't quantified in this work that also exert influence in the cultivars' antioxidant activity.

4.2.2. DPPH● Assay

DPPH is an easy and quick method to determine antioxidant capacity through hydrogen donation. This synthetic free radical is also stable because unlike other free radicals this molecule is not dimerized. There is simply an electron delocalization, which is also responsible for the characteristic violet colour. When mixed with a substrate with radical scavenging activity DPPH• is reduced and there is a loss of the violet colour proportional to the antioxidant power of the substrate (63) This assay is widely used due to its stability and simple procedure, and it has a reasonable cost. It gains its importance being the most direct way of measuring an extract's hydrogen or electron donation ability to the synthetic DPPH (63,113). All extracts exhibited antioxidant activity in a concentration dependent-manner (Figure 4). *Royal Lu* was the most active with an IC_{50} = 62.0 ±1.5 µg/mL, followed by *Sweet Dreams* (IC_{50} = 65.1 ± 2.5 µg/mL) and *Fidelia* (IC_{50} = 79.0 ± 1.4 µg/mL) (Figure 8 and Table 4). All extracts were better active than positive control ascorbic acid (IC_{50} = 18.9 ± 0.2 µg/mL) (Table 4). Royal Lu's activity was approximately three times superior to that of the ascorbic acid, and the least active cultivar *Summer Rich* (IC_{50} = 146.8 ± 1.4) was seven times higher than positive control.

Table 4. IC_{50} (µg/mL) values found in the antioxidant activity and α-glucosidase assays for peach extracts.

Assay	*Summer Rich*	*Fidelia*	*Royal Glory*	*Royal Magister*	*Royal Lu*	*Sweet Dreams*
DPPH•	146.7 ± 1.4	79.1 ± 1.4^{a}	$102.2 \pm 1.5^{a,b}$	$98.4 \pm 2.7^{a,b}$	$62.1 \pm 1.5^{a,b,c,d}$	$65.1 \pm 2.5^{a,b,c,d}$
•NO	2754.1 ± 236.9	1799.1 ± 101.1^{a}	1735.1 ± 261.5^{a}	$2606.9 \pm 66.2^{a,bc}$	$1935.0 \pm 218.4^{a,d}$	$421.2 \pm 45.4^{a,b,c,d,e}$
α-glucosidase	35.8 ± 2.5	17.1 ± 1.7^{a}	15.0 ± 1.1^{a}	11.7 ± 1.4^{a}	$25.1 \pm 1.1^{a,b,c,d}$	$20.3 \pm 2.6^{a,d}$

Values are expressed as mean ± standard deviation of three assays; difference between each activity for the tested extracts were tested for significance using the one way analysis of variance (ANOVA) with the post-hoc LSD test.

Extracts from *P. persica* have been subjected to testing for free radical scavenging activity using the DPPH radical method before. A study compared various fruits from the *Rosaceae* family which included plum, peach and cherry, expressing DPPH• scavenging activities of IC_{50} = 17.5 ± 0.9 µg/mL, IC_{50} = 18.2 ± 0.7 µg/mL and IC_{50} = 16.2 ± 0.2 µg/mL expressed as dried fruit extract, respectively (118). This difference from this peach activity towards our samples can be due to many factors like phenolic profile or the time of harvest, since ripe fruits have less antioxidant content. This same effect can be seen in other plants, like garlic (119). . Comparing our results with another study assessing the blackberry's fruit extract DPPH• scavenging activity we can see that some of our samples have higher scavenging potential, with blackberry's activities ranging from IC_{50} = 96.0 ± 0.3 µg/mL to IC_{50} = 118.1 ± 0.1 µg/mL, being that the extract with most activity had an increased sonication time and/or temperature which might increase the total anthocyanin content in the extract since blackberries are rich in this class of flavonoids (116). This activity has also been assessed for individual phenolic compounds, like chlorogenic acid, which was identified in all cultivars (Table 2), has shown activity against DPPH•. Hydroxybenzoic acids like gallic acid and protocatechuic acid have been identified in peach in previous studies, and their antioxidant activity has also been tested against DPPH• (120). Comparing the results obtained with literature data, our samples demonstrate a good antioxidant activity (118,119,121).

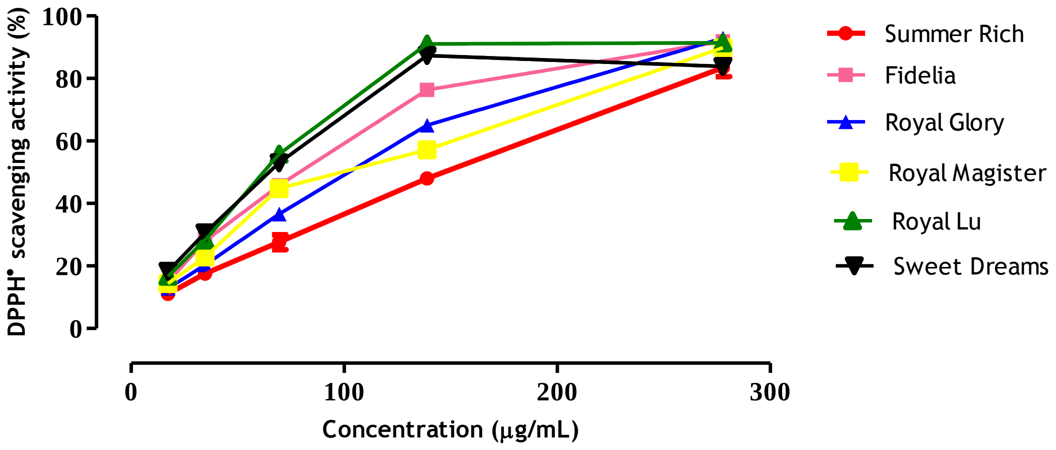


Figure 8. Scavenging activity of peach extracts against DPPH•.

As seen in the determined phenolic profile, *Royal Lu* is the richest cultivar in the study. Even though it has low levels of 3-*O*-caffeoylquinic and 5-*O*-caffeoylquinic acids it compensates with high hydroxybenzoic acid levels (Table 3). Hydroxycinnamic acids are usually better hydrogen donators due to their CH=CH-COOH group. However, if this hydroxybenzoic acid derivative is either a trihydroxybenzoic or dihydroxybenzoic acid this high antioxidant activity is justifiable. In this case the number and position of hydroxyl groups is the key factor influencing antioxidant activity (54,72). Besides this, *Royal Lu* is the only cultivar in the study with ferulic acid in its composition (Table 3) and is also rich in catechins. As for *Sweet Dreams*, this one is both rich in 3-*O*-caffeoylquinic and 5-*O*-caffeoylquinic acids, catechins and quercetin-3-*O*-rutinoside. Finally, the *Fidelia* cultivar owes its antioxidant power mainly to chlorogenic and neochlorogenic acids. *Summer Rich* is the less active because it is relatively poor compared to the other remaining cultivars. Besides the hydroxycinnamic acids, both *Royal Glory* and *Royal Magister* are richer in flavan-3-ols and flavonols giving them structural advantages in antioxidant activity (72).

4.2.3. Nitric oxide

Nitric oxide is a RNS generated by specific nitric oxide synthases that metabolize arginine to citruline with formation of $^{\bullet}$NO. The inducible type, iNOS, is expressed in inflammation during which ROS and NO anions are produced by activated phagocytes and macrophages, respectively. The toxicity of $^{\bullet}$NO increases when it reacts with superoxide to form the peroxynitrite anion ($ONOO^-$). Peroxynitrite oxidizes LDL directly, causing irreversible damage to cell membranes. This inflammatory consequence can be diminished by flavonoids' capacity to scavenge free radicals like $^{\bullet}$NO and preventing it from reacting with other radicals, thus reducing damage to cells (64-67,113). In this assay sodium nitroprusside is decomposed in aqueous solution at physiological pH (7.2) producing $^{\bullet}$NO.

Concerning nitric oxide scavenging, peaches extracts showed notable concentration-dependent effects (Figure 9). *Sweet Dreams* was the most active (IC_{50} = 421.2 ± 45.5 µg/mL) followed by *Royal Glory* (IC_{50} = 1735.1 ± 261.5) and the less active cultivar was *Summer Rich* (IC_{50} = 2754.0 ± 236.9 µg/mL) (Figure 9 and Table 4). These results are much lower compared with positive control ascorbic acid (IC_{50} = 67.6 ± 7.4 µg/mL).

Comparatively to previous data regarding NO scavenging activity, the activity measured for the present hydroethanolic peach extracts were not as promising as those found for cherry or for cranberry flavonoids, presenting values of IC_{50} =22.8 ± 2.5 µg/mL fruit extract and IC_{50} = 4.4 ± 0.4 µg/mL of dried cranberry flavonoid extract, respectively. This big difference is probably due the fruits' high content in anthocyanins (122,123).

Phenolic compounds extracted from crude drugs were reported to scavenge nitric oxide, highlighting catechin (IC_{50} = 257.4 ± 5.1 µg/mL) which was reported in the peaches' phenolic profiles. Comparing with the nitric oxide scavenging activity reported for epicatechin-3-*O*-gallate (IC_{50} =120.9 ± 1.5 µg/mL), we can see the influence of structural differences(124). This activity reflects the esterification with gallic acid and its contribution to the scavenging activity (72,124).

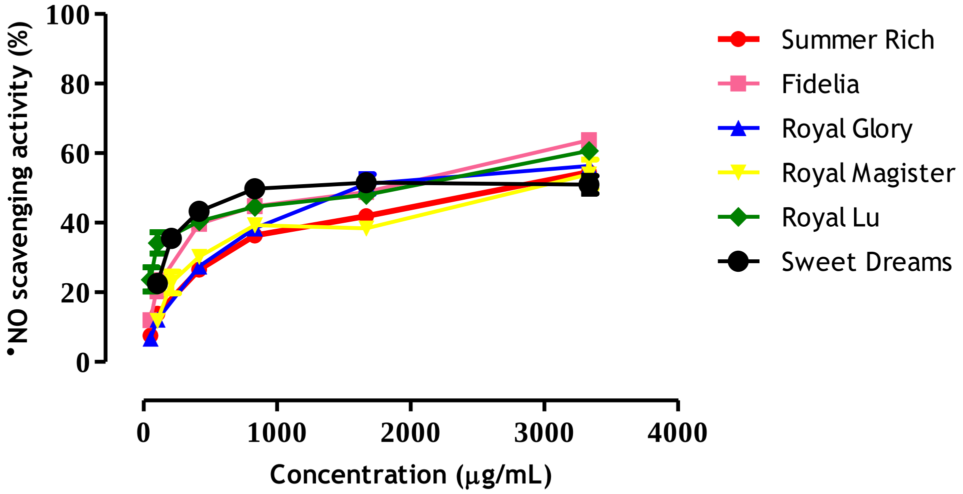


Figure 9. Scavenging activity of peach extracts against •NO.

Comparing to the other cultivars Sweet Dreams is the richest in chlorogenic acid and catechin. Like it was previously mentioned, hydroxycinnamic acids like chlorogenic acid are good antioxidants due to their number of hydroxyl groups (OH) and their carboxyl group (CH=CH-COOH). Catechin, on the other hand, lacks one of the key structural features that confer flavonoids great antioxidant power. Catechin is missing the 2,3-double bond and the 4-*oxo* group on C ring preventing the electron transfer from B ring to A ring. This is an important feature since it is what stabilizes the aryloxyl radical after the hydrogen donation.

Other cultivars such as Royal Glory and Royal Magister are rich in quercetin, cyanidin-3-*O*-glucoside and catechin derivatives. Quercetin possesses one of the highest radical scavenging activities due the its structure: five hydroxyl groups (including the catechol group in B ring), the 2,3-double bond and 4-*oxo* group in C ring. Cyanidin has a similar structure with the same number of hydroxyl groups and unsaturated heterocyclic C ring, thus possessing a similar TAEC (72). Catechin derivatives such as epigallocatechin or catechin gallates are also known to possess higher antioxidant activity than catechin, but until now it seems to be only detected in peach seed (125). Nevertheless, the overall antioxidant effect might be greater since these compounds are able to prevent other radicals from reacting with nitric oxide, making them effective in preventing pathological processes like cellular damage by LDL oxidation, tumour initiation, promotion and progression, cardiovascular or neurodegeneration derived from inflammation. Also the presence of other not identified compounds cannot be ignored (54,67,71,72,115,125).

4.3. α-glucosidase inhibitory activity

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycaemia accompanied by disturbances in carbohydrate, fat and protein metabolisms due to inadequate secretion of insulin, insulin's action or both (126,127). This hyperglycaemia is the hallmark of DM and brings consequences such as long-term damage, dysfunction and failure of various organs like the eyes, kidneys, testicles, brain, nerves, heart, and blood vessels (127,128).The α-glucosidase enzyme is an important factor in carbohydrate digestion and it has been targeted as a means to control and lower postprandial hyperglycaemia slowing down the development of diabetes and helping the noninsulin-dependent type 2 diabetes patients control their levels, being postprandial hyperglycaemia the first metabolic abnormality manifestation (77).

In the present work and to our knowledge, we tested for the first time the α-glucosidase inhibitory effect of the peach extracts. All extracts demonstrated inhibitory activity towards the α-glucosidase enzyme in a concentration dependent manner. The cultivar with most inhibitory capacity was *Royal Magister* (IC_{50} = 11.7 ± 1.4 μg/mL), followed by *Royal Glory* (IC_{50} = 15.0 ± 1.2 μg/mL). *Summer Rich* was the cultivar with less activity (IC_{50} = 35.8 ± 2.5 μg/mL of extract) (Figure 10, Table 4). In comparison to the therapeutic drug acarbose (IC_{50} = 306.7 ± 2.1 μg/mL), the extracts of the peach samples presented very good inhibitory activity, being 8.5 to 26 times inferior to the one achieved by the positive control acarbose. These results are promising, showing that some cultivars are more active than others and good inhibitory activity can be achieved with these extracts.

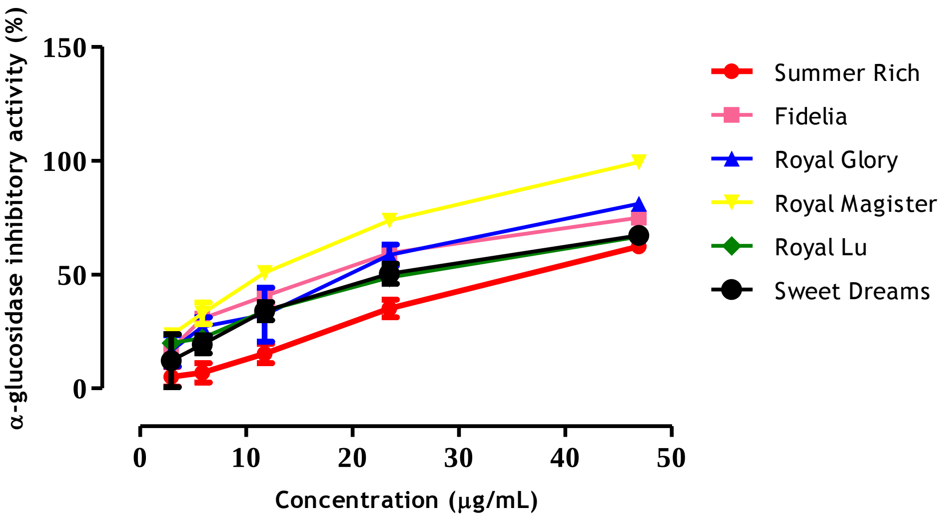


Figure 10. Inhibitory activity peach extracts on α-glucosidase.

The peach extracts showed considerable better activity than those obtained with other fruits and plants used in the treatment of diabetes, namely plum (*Davidsonia pruriens*) and quandong (*Santalum acuminatum*) with IC_{50} = 130 ± 1 µg/mL and IC_{50} = 390 ± 10 µg/mL expressed as dried fruit extract, respectively, and strawberries (IC_{50} = 50 µg/mL expressed as dried fruit extract) (129). Medicinal plants have been combined for diabetes therapy purposes, making four combined aqueous extracts of *Stevia rebaudiana, Momordicha charantia, Tamarindus indica, Gymnema sylvestre, Allium sativum and Murraya koenigii*. These four combinations presented good inhibitory activity (IC_{50} ranged from 38.1 ± 2.3 µg/mL to 91 ± 1.8 µg/mL expressed as dried extract), still the inhibitory activity achieved with peach extracts are almost 3 times higher than the ones reported for the medicinal plants aqueous extracts (130).

According to information stated on previous literature, the antidiabetic activity can be due to the presence of phenolic acids like chlorogenic acid and flavonoids such as catechin, quercetin and specially tannins and anthocyanins (129) A study using pure commercial compounds tested their inhibitory activity of α-glucosidase enzyme, the values presented herein for compounds present in peach were as follows: 91% inhibition by quercetin, 45% inhibition by catechin and 99% inhibition by cyanidin at a concentration of 200 µM (131). This is concurrent with the inhibitory activities obtained for the peach cultivars. Observing the activities presented by the three most active cultivars, *Royal Magister*, followed by *Royal Glory*, we can see that these have the highest contents of anthocyanins 153.5 µg/g and 54.3 µg/g of dried fruit, respectively (Table 4). As for the third most active, *Fidelia* has a low anthocyanin content (2.7 µg/g) but the non-coloured profile is rich in hydroxycinnamic acids that represent 40% of total non-coloured phenolics present in *Fidelia* (Table 4) (129,131). Previous literature reports results for phenolic compounds extracted from the flower *Edgeworthia gardneri*. The results showed a weak inhibitory activity for both hydroxybenzoic acids and hydroxycinnamic acids like 4-hydroxybenzoic acid (IC_{50} = 1200 ± 134 µg/mL), ferulic acid (IC_{50} > 2000 µg/mL) and caffeic acid (IC_{50} = 957 ± 36 µg/mL), and reported the flavonols quercetin (IC_{50} = 5.1 ± 0.3 µg/mL) and kaempferol (IC_{50} = 56.2 ± 4.1 µg/mL) as the most active α-glucosidase inhibitors (132).

4.4. Protective effect of peach extract against ROO$^{\bullet}$-induced oxidative damage in human erythrocytes

4.4.1. Inhibition of hemolysis

Erythrocytes' membranes are fatty acid rich structures, susceptible to lipid peroxidation mediated by ROS and RNS. These reactions can occur due to oxygen transport by redox active hemoglobin, or they can take place when an imbalance between oxidants and antioxidants occurs. For instance, an exacerbated inflammatory response results in production of ROS and $^{\bullet}NO$. This production of peroxynitrite ($ONOO^{-}$) will directly oxidize LDL and cause irreversible damage to membranes (41,61,66,67,98). Such consequences can be attenuated or avoided by phenolic compounds. Their antioxidant activity is usually associated to activities like free-radical scavenging, electron donation and metal-chelating properties (54). However, it was reported that some of these phenolic compounds can interact with the bilayer decreasing its fluidity and consequently decreasing diffusion of free radicals into the membrane preventing their possible damage (133).

In this study we report for the first time, the protective effect of peach cultivar *Royal Lu* extract against peroxyl radical induced hemolysis, which was assessed by inducing an environment of oxidative stress. This was achieved with peroxyl radical initiator AAPH. The thermal decomposition of the initiator at physiological temperature (37ºC) generates $ROO^{\bullet}$ in aqueous phase. Then the membrane is quickly damaged due to the chain reaction (98,134,135). The peach extract inhibited hemolysis in a concentration-dependent manner with an IC_{50} value at 109.9 ± 4.5 µg/mL (Figure 11), but it is not as efficient as quercetin (IC_{50} = 0.7 µg/mL) (97).

Comparing with strawberry extract, Royal Lu extract showed an activity 4 times higher (IC_{50} = 430 ± 91 µg/mL) expressed as dried fruit extract), despite this one being a fruit known for its rich content in antioxidants. On the other hand, *Arbutus unedo* L leaf extract showed a much higher protective potential (IC_{50} = 62 ± 2 µg/mL expressed as dried leaf extract) (136). Concurrent with these, *Cydonia oblonga* Miller(quince) fruit extract had an activity 6 times lower than the Royal Lu extract (IC_{50} = 652 µg/mL expressed as dried extract), but as for the leaf extract activity (IC_{50} = 30.7 ± 6.7 µg/mL) it was almost 4 times more active than our extract (133,137). Green tea is long known for its antioxidant properties and other health benefits, the antihemolytic activity measured for the leaves was 4 times higher (IC_{50} = 24.3 ± 96 µg/mL expressed as dried extract) than that of peach extract determined in this work (133).

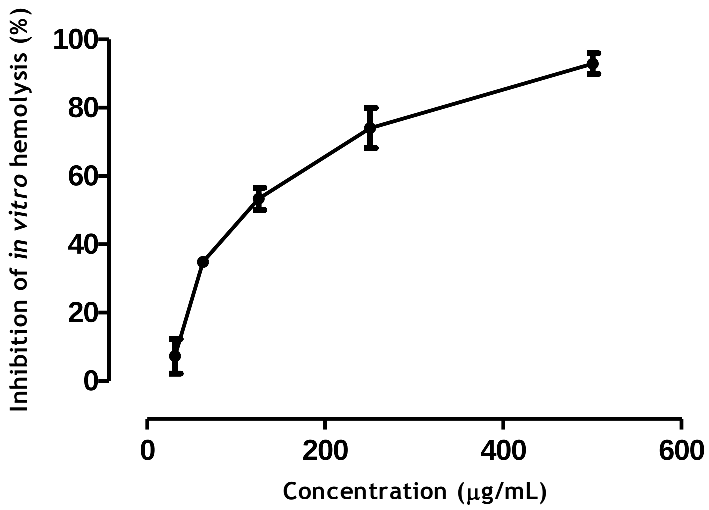


Figure 11. Hemolysis inhibition (%) by Prunus persica Royal Lu extract.

This inhibitory feature is of course associated with the phenolic compounds radical scavenging activity. They scavenging the ROO$^\bullet$ that attack the membrane bilayer of erythrocytes and prevent the free-radical chain reaction and therefore prevent hemolysis. There are no previous studies reporting this activity in peach, but there are some reporting the same activity for the pure phenolic compounds (138,139). Natella and collaborators decided to directly test the antioxidant activity of a number of pure compounds by measuring the formation of conjugated diene hydroperoxides that are produced by AAPH. They used two synthetic antioxidants as comparing method. All the compounds except for ferulic acid surpassed the synthetic controls. Caffeic and 5-*O*-caffeoylquinic acid showed the same activity, 40 min/µM. As for flavonoids, they all had high activity according to their chemical structure mentioned. Quercetin had the highest activity (55 min/µM), followed by epigallocatechin (44 min/µM), rutin (41 min/µM), epicatechin (41 min/µM) and catechin (33 min/µM) (139). Additionally, anthocyanins have also been able to successfully inhibit hemolysis in a concentration-dependant manner (140).This activity is in accordance to their structure, namely number of hydroxyl groups, the 4 *oxo* group and the C2=C3 in C ring that has shown to be important in flavonoids antioxidant activity (72). This particular cultivar in study is not rich in cinnamic acids, in fact *Royal Lu* is composed mainly by hydroxybenzoic acids (88,7%) and only 5,59% of flavan-3-ols. According to these studies, benzoic acids are the least active (138,139). Although, the high amount of this compound present in this cultivar and thus the amount of hydroxyl groups available must be taken in consideration. This is also the same cultivar that had the strongest activity against DPPH• (IC_{50} = 62.0 ±1.5 µg/mL), therefore this inhibitory activity against peroxyl radical induced hemolysis is in accordance with the DPPH assay results, as previously reported by Magalhães *et al.* (2009) that found the same correlation (137). However, the presence in the extract of other non-identified bioactive compounds cannot be ignored, like carotenoids reported in peach, that were reported to possess capacity for hemolysis inhibition (11,141,142).

4.4.2. Inhibition of hemoglobin oxidation

Hemoglobin is a hemeprotein responsible for carrying oxygen from the lungs to the tissues, and is one of the least recognized causes of cellular damage when subjected to oxidation. Several pathologies like thalassemias, sickle cell disease, hemolytic anemia, iron-deficiency anemia and repeated blood donations usually end up causing oxidative denaturation of hemoglobin and, after hemolysis, the release of the denaturated products into circulation. Extracellular hemoglobin is a nitric oxide scavenger and rapidly oxidizes to methaemoglobin. This intensifies inflammatory responses in the vessels' endothelial cells promoting LDL oxidation which leads to atherosclerosis (143).

To study the Royal Lu's ability to inhibit hemoglobin oxidation peroxyl radicals were generated by AAPH thermal decomposition to induce hemoglobin oxidation. $ROO^{\bullet}$ react with oxyhaemoglobin and the end product of the reaction is methaemoglobin. The extract's inhibitory capacity was determined for the first time by monitoring the rate of methaemoglobin formation at 630 nm. *Royal Lu* peach extract was also able to inhibit hemoglobin oxidation in a dose-dependent manner with an IC_{50} at 83.8 ± 6.5 µg/mL (Figure 12). Comparing our results with previous study using murici fruit extract we can see that the peach had 3 times higher activity preventing hemoglobin oxidation than the murici extract (142). This activity can be explained by this cultivars high content on hydroxybenzoic derivative, which is supported by a previous study reporting an activity of IC_{50} at 12.0 ± 0.1 µg/mL for gallic acid (141).

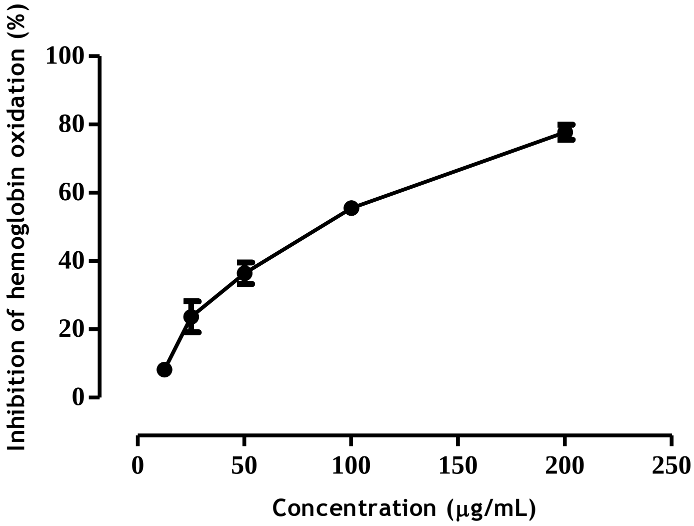


Figure 12. Inhibition of hemoglobin oxidation (%) by *Prunus persica* Royal Lu extract.

Studies have been made assessing the protective effects of phenolics on hemolysis, but none of them explores the protective action against hemoglobin oxidation. As it was mentioned before for the inhibitory activity against peroxyl radical induced hemolysis, hemoglobin oxidation is likely due to the phenolic profile of *Royal Lu*, rich in hydroxybenzoic acids. Despite not being considered the most active by some studies, the amount and number of hydroxyl groups is important in electron donation and must be taken in account (72,138,139). Along with this the small amounts of flavan-3-ols (5.59% of total non-coloured phenolics), quercetin (0.17% of total non-coloured phenolics), and cyanidin-3-O-glucoside (85% of its total anthocyanin content) also have to be taken in consideration. Not forgetting the phytochemicals present in the fruit that were not quantified in this work, such as carotenoids that can inhibit lipid peroxidation and hemoglobin oxidation in human erythrocytes (141).

5. Conclusions

The results obtained in this thesis led to the following conclusion:

- The analysis performed by LC-DAD of peach samples allowed the identification of a total of 17 phenolic compounds, that includes three anthocyanins and fourteen non-coloured phenolics. Cyanidin-3-*O*-glucoside was the main anthocyanin present in all six cultivars, while regarding the non-coloured phenolics, phenolic acids were found in higher amounts, in particular the 3-*O*-caffeoylquinic acid 5-*O*-caffeoylquinic acid with exception of one cultivar, *Royal Lu*, for which the main compound was a hydroxybenzoic acid derivative..
- *Royal Lu* was the richest in non-coloured phenolics. Regarding the anthocyanin, Royal Magister revealed the highest contents.
- In a general way the cultivars showing the highest antioxidant activity were *Royal Lu* and *Sweet Dreams*. Both cultivars had a good antioxidant activity in FRAP and radical scavenging activity against DPPH• .On the other hand *Sweet Dreams* was also the most active against nitric oxide radical. This antioxidant activity is mainly due to their phenolic contents, emphasizing phenolic acids and considerable amounts of cyanidin-3-*O*-glucoside both with high hydrogen donating capacity due to their structures rich in hydroxyl groups.
- *Royal Magister* and *Royal Glory* showed the best inhibitory activity against the α-glucosidase enzyme, showing far superior results than those achieved by the therapeutic drug acarbose used as a positive control.
- *Royal Lu* extract was able to successfully inhibit hemolysis and hemoglobin oxidation in human erythrocytes being the richest cultivar in phenolic compounds it has a high antioxidant power, easily scavenging the peroxyl radicals that attack the bilayer membrane of erythrocytes. Moreover, it was reported previously that some phenolic compounds have the ability of interacting with the bilayer reducing the membrane's fluidity and thus preventing the free radical diffusion that damages the cell.
- In an overall, the results obtained in the present work lead us to conclude that the peach has a great biological potential. Its extracts demonstrated a great antioxidant capacity by scavenging free radicals, being able to protect cells against oxidative stress and consequent damages. This can be extrapolated as numerous health benefits specialy against inflammation, atherosclerosis, cardiovascular diseases, diabetes, hemolytic pathologies, cardiovascular, neurodegenerative and oncological disease prevention. Still, more studies should be conducted to further explore the peach's phytochemical profile and biological potential and respective benefits of their consumption to human health.

6. References

1. Del Rio D, Rodriguez-Mateo A, Spencer JPE, Tognolini M, Borges G, Crozier A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid Redox Signal. 2012;18(14):1818–92.

2. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep. 2009;26(8):1001–43.

3. Abidi W, Jiménez S, Moreno MÁ, Gogorcena Y. Evaluation of antioxidant compounds and total sugar content in a nectarine [Prunus persica (L.) Batsch] progeny. Int J Mol Sci. 2011;12(10):6919–35.

4. Agudo A. Measuring intake of fruit and vegetables. FAO/WHO Workshop on Fruit and Vegetables for Health. Kobe, Japan: World Health Organization; 2005.

5. Cantín CM, Moreno M a., Gogorcena Y. Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine [Prunus persica (L.) batsch] breeding progenies. J Agric Food Chem. 2009;57(11):4586–92.

6. Davidović SM, Veljović MS, Pantelić MM, Baošić RM, Natić MM, Dabić DČ, et al. Physicochemical, antioxidant and sensory properties of peach wine made from Redhaven cultivar. J Agric Food Chem. 2013;61(6):1357–63.

7. Faust M, Timon B. Origin and Dissemination of peach. Hortic Rev (Am Soc Hortic Sci). 1995;17:331–79.

8. Campbell OE, Padilla-Zakour OI. Phenolic and Carotenoid Composition of Canned Peaches (Prunus Persica) and Apricots (Prunus Armeniaca) as Affected by Variety and Peeling. Food Res Int [Internet]. Elsevier Ltd; 2013;54(1):448–55. Available from: http://dx.doi.org/10.1016/j.foodres.2013.07.016

9. Cantín CM, Gogorcena Y, Moreno MÁ. Phenotypic diversity and relationships of fruit quality traits in peach and nectarine [Prunus persica (L.) Batsch] breeding progenies. Euphytica. 2009;171(2):211–26.

10. Liu H, Cao J, Jiang W. Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars. LWT - Food Sci Technol [Internet]. Elsevier Ltd; 2015;63(2):1042–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0023643815003345

11. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Kader AA. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. J Agric Food Chem. 2002;50(17):4976–82.

12. Liu H, Cao J, Jiang W. Evaluation of physiochemical and antioxidant activity changes during fruit on-tree ripening for the potential values of unripe peaches. Sci Hortic

(Amsterdam). Elsevier B.V.; 2015;193:32–9.

13. Zhao X, Zhang W, Yin X, Su M, Sun C, Li X, et al. Phenolic Composition and Antioxidant Properties of Different Peach [Prunus persica (L.) Batsch] Cultivars in China. Int J Mol Sci. 2015;16(3):5762–78.

14. Colaric M, Veberic R, Stampar F, Hudina M. Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. J Sci Food Agric. 2005;85(April):2611–6.

15. Versari A, Castellari M, Parpinello GP, Riponi C, Galassi S. Characterisation of peach juices obtained from cultivars Redhaven, Suncrest and Maria Marta grown in Italy. Food Chem. 2002;76(2):181–5.

16. Wang Y, Yang C, Li S, Yang L, Wang Y, Zhao J, et al. Volatile characteristics of 50 peaches and nectarines evaluated by HP-SPME with GC-MS. Food Chem. Elsevier Ltd; 2009;116(1):356–64.

17. Byrne DH, Nikolic AN, Burns EE. Variability in Sugars, Acids, Firmness, and Color Characteristics of 12 Peach Genotypes. J Am Soc Hortic Sci. 1991;116(6):1004–6.

18. Reig G, Iglesias I, Gatius F, Alegre S. Antioxidant capacity, quality, and anthocyanin and nutrient contents of several peach cultivars [Prunus persica (L.) Batsch] grown in Spain. J Agric Food Chem. 2013;61(26):6344–57.

19. Ribeiro RA, Miranda GV, Puiatti M. Amido Nos Grãos Verdes De Cultivares De Milho Na Colheita Study of Total Soluble Sugars , Reducing Sugars and Starch Composition in Immature Kernels of Corn Cultivars. 2004;

20. Oliveira MADE, Cereda MP, Cabello C, Urbano LH. Quantificação de açúcares em pêssegos da variedade “Biuti”, armazenados sob cndições de ambiente e refrigeração. Rev Bras Frutic Jaboticabal. 2001;23:424–7.

21. Sánchez-Moreno C, Pascual-Teresa S De, Ancos B De, Cano MP. Nutritional Values of Fruits. Handbook of FRuits and Fruit Processing. Blackwell Publishing; 2006. p. 29–44.

22. Siddiq M. Peach and Nectarine. Handbook of Fruits and Fruit Processing. Blackwell Publishing; 2006.

23. USDA. National Nutrient Database for Standard Reference [Internet]. National Agricultural Library. 2015. Available from: https://ndb.nal.usda.gov/ndb/search

24. Duan Y, Dong X, Liu B, Li P. Relationship of changes in the fatty acid compositions and fruit softening in peach (Prunus persica L. Batsch). Acta Physiol Plant. 2013;35(3):707–13.

25. Kikalishvili Bi, Zurabashvili D, Turabelidze D, Zurabashvili Z, Giorgobiani I. The fatty acid composition of peach oil and its biological activity. Georg Med News. 2013;218:82–5.

26. Alina O, Alexa E, Radulov I, Costea A, Dobrei A, Dobrei A. Minerals and Amino Acids in Peach (Prunus persica L .) Cultivars and Hybrids Belonging to World Germoplasm Collection in the Conditions of West Romania. 2015;6:145–50.

27. Kim H-R, Kim I-D, Dhungana SK, Kim M-O, Shin D-H. Comparative assessment of physicochemical properties of unripe peach (Prunus persica) and Japanese apricot (Prunus mume). Asian Pac J Trop Biomed. 2014;4(2):97–103.

28. Buedo AP, Elustondo MP, Urbicain MJU. Amino acid loss in peach juice concentrate during storage. 2001;281–8.

29. Jones D. Organic acids in the rhizosphere - a critical review. Plant Soil. 1998;205(1):25–44.

30. INSA. PORTAL DO UTENTE PORTAL DA SAÚDE Detalhe Alimento [Internet]. 2015 [cited 2015 Aug 15]. p. 2–3. Available from: www.insa.pt

31. Başar H. Elemental composition of various peach cultivars. Sci Hortic (Amsterdam). 2006;107(3):259–63.

32. Melo GWB de, Sete PB, Ambrosini VG, Freitas RF, Basso A, Brunetto G. Nutritional status, yield and composition of peach fruit subjected to the application of organic compost. Acta Sci Agron. 2016;38(1):103.

33. Manzoor M, Anwar F, Mahmood Z, Rashid U, Ashraf M. Variation in minerals, phenolics and antioxidant activity of peel and pulp of different varieties of peach (Prunus persica L.) fruit from Pakistan. Molecules. 2012;17(6):6491–506.

34. Anderson JJB, Garner SC. Calcium and Phosphorus in Health and Disease. CRC Press; 1995. 2 p.

35. Rasool Hassan BA. Vitamins (Importance and Toxicity). Pharm Anal Acta. 2012;3(8).

36. Ozkanlar S, Akcay F. Antioxidant vitamins in atherosclerosis--animal experiments and clinical studies. Adv Clin Exp Med. 2012;21(1):115–23.

37. Visai C, Vanoli M. Volatile compound production during growth and ripening of peaches and nectarines. Sci Hortic (Amsterdam). 1997;70(1):15–24.

38. El Hadi M, Zhang F-J, Wu F-F, Zhou C-H, Tao J. Advances in Fruit Aroma Volatile Research. Molecules. 2013;18(7):8200–29.

39. Jiang Y, Song J. Volatile compounds and their biological characteristic of major fruits. In: Hui IH, editor. Handbook of Fruits and Vegetable Flavors. Hoboken, New Jersey: John Wiley and Sons, Inc.; 2010. p. 13.

40. Dragovic-Uzelac V, Levaj B, Mrkic V, Bursac D, Boras M. The content of polyphenols and carotenoids in three apricot cultivars depending on stage of maturity and geographical region. Food Chem. 2007;102(3):966–75.

41. Gasparotto J, Somensi N, Bortolin RC, Girardi CS, Kunzler A, Rabelo TK, et al.

Preventive supplementation with fresh and preserved peach attenuates CCl4-induced oxidative stress, inflammation and tissue damage. J Nutr Biochem. Elsevier Inc.; 2014;25(12):1282–95.

42. Vizzotto M, Cisneros-zevallos L, Byrne DH. Large Variation Found in the Phytochemical and Antioxidant Activity of Peach and Plum Germplasm. J Am Soc Hortic Sci. 2007;132(3):334–40.

43. Ignat I, Volf I, Popa VI. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem. Elsevier Ltd; 2011;126(4):1821–35.

44. Remorini D, Tavarini S, Degl'Innocenti E, Loreti F, Massai R, Guidi L. Effect of rootstocks and harvesting time on the nutritional quality of peel and flesh of peach fruits. Food Chem. 2008;110(2):361–7.

45. Drogoudi PD, Tsipouridis CG. Effects of cultivar and rootstock on the antioxidant content and physical characters of clingstone peaches. Sci Hortic (Amsterdam). 2007;115(1):34–9.

46. Silva L, Costa R. Health benefits of nongallated and gallaed flavan.3-ols: A prospectus. Annu Rev Nutr. 2014;20(1):561–93.

47. Monagas M, Bartolomé B, Gómez-Cordovés C. Updated knowledge about the presence of phenolic compounds in wine. Crit Rev Food Sci Nutr. 2005;45(2):85–118.

48. Sun L, Zhang H, Zhuang Y. Preparation of Free, Soluble Conjugate, and Insoluble-Bound Phenolic Compounds from Peels of Rambutan (Nephelium lappaceum) and Evaluation of Antioxidant Activities in vitro. J Food Sci. 2012;77(2):198–204.

49. Nicoletti I, Martini D, De Rossi A, Taddei F, D'Egidio MG, Corradini D. Identification and quantification of soluble free, soluble conjugated, and insoluble bound phenolic acids in durum wheat (triticum turgidum L. var. durum) and derived products by RP-HPLC on a semimicro separation scale. J Agric Food Chem. 2013;61(48):11800–7.

50. Acosta-Estrada B a., Gutiérrez-Uribe J a., Serna-Saldívar SO. Bound phenolics in foods, a review. Food Chem. Elsevier Ltd; 2014;152:46–55.

51. Häkkinen S. Flavonols and Phenolic Acids in Berries and Berry Products Flavonols and Phenolic Acids in Berries and Berry Products. University of Kuopio; 2000.

52. Ceccarelli D, Simeone AM, Nota P, Piazza MG, Fideghelli C, Caboni E. Phenolic compounds (hydroxycinnamic acids, flavan-3-ols, flavonols) profile in fruit of Italian peach varieties. Plant Biosyst - An Int J Deal with all Asp Plant Biol. 2016;3504(February):1-6.

53. Aherne SA, O'Brien NM. Dietary flavonols: chemistry, food content, and metabolism. Nutrition. 2002;18(1):75–81.

54. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99(1):191–203.

55. Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M, Sharma P. A Review of Phytochemistry and Pharmacology of Flavonoids. Int Pharm Sci. 2011;1(1):25–41.

56. Škerget M, Kotnik P, Hadolin M, Hraš AR, Simonič M, Knez Ž. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem. 2005;89(2):191–8.

57. Hollman PCH, Katan MB. Dietary flavonoids: Intake, health effects and bioavailability. Food Chem Toxicol. 1999;37:937–42.

58. Puupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia a., et al. Antimicrobial properties of phenolic compounds from berries. J Appl Microbiol. 2001;90(4):494–507.

59. Flamini R, Mattivi F, De Rosso M, Arapitsas P, Bavaresco L. Advanced knowledge of three important classes of grape phenolics: Anthocyanins, stilbenes and flavonols. Int J Mol Sci. 2013;14(10):19651–69.

60. Aron PM, Kennedy JA. Flavan-3-ols: Nature, occurrence and biological activity. Mol Nutr Food Res. 2008;52(1):79–104.

61. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. HortScience. 2000;35(4):588–92.

62. Freeman B, Crapo J. No TitleBiology of disease: free radicals and tissue injury. Lab Investig. 1982;47(5):412–26.

63. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J. 2013;21(2):143–52.

64. Kong J. Analysis and biological activities of anthocyanins. Phytochemistry. 2003;64(5):923–33.

65. Wallace TC. Anthocyanins in Cardiovascular Disease 1. Adv Nutr. 2011;2(7):1–7.

66. Kim HP, Son KH, Chang HW, Kang SS. Critical Review Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. J Pharmacol Sci. 2004;96:229–45.

67. García-Lafuente A, Guillamón E, Villares A, Rostagno M a., Martínez JA. Flavonoids as anti-inflammatory agents: Implications in cancer and cardiovascular disease. Inflamm Res. 2009;58(9):537–52.

68. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002;82(1):47–95.

69. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2(4):152–9.

70. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002;13(10):572–84.

71. Williams RJ, Spencer JP., Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med. 2004;36(7):838–49.

72. Rice-Evans C a., Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933–56.

73. Gasparotto J, Somensi N, Bortolin RC, Moresco KS, Girardi CS, Klafke K, et al. Effects of different products of peach (Prunus persica L. Batsch) from a variety developed in southern Brazil on oxidative stress and inflammatory parameters in vitro and ex vivo. J Clin Biochen Nutr. 2013;52(May):202–7.

74. Yao X-C, Cao Y, Wu S-J. Antioxidant activity and antibacterial activity of peach gum derived oligosaccharides. Int J Biol Macromol. Elsevier B.V.; 2013;62:1–3.

75. Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. J Diabetes Metab Disord. 2013;12(1):43.

76. Kozłowska A, Szostak-Wegierek D. Flavonoids--food sources and health benefits. Rocz Pańtwowego Zakładu Hig. 2014;65(2):79–85.

77. Nasu R, Miura M, Gomyo T. Effects of fruit, spices and herbs on alpha glucosidase activity and glycemic index. Food Sci Technol Res. 2005;11(1):77–81.

78. Hoek M van. Cardiovascular disease prevention: Mind the gap…. Neth J Med. 2015;73(7):308–9.

79. Lee CH, Kim JH. A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. J Ginseng Res. Elsevier Ltd; 2014;38(3):161–6.

80. Lavie CJ, De Schutter A, Parto P, Jahangir E, Kokkinos P, Ortega FB, et al. Obesity and Prevalence of Cardiovascular Diseases and Prognosis – the Obesity Paradox Updated. Prog Cardiovasc Dis. Elsevier Inc.; 2016;1–11.

81. Loh WM, Ling WC, Murugan DD, Lau YS, Achike FI, Vanhoutte PM, et al. Des-aspartate angiotensin I (DAA-I) reduces endothelial dysfunction in the aorta of the spontaneously hypertensive rat through inhibition of angiotensin II-induced oxidative stress. Vascul Pharmacol. Elsevier Inc.; 2015;71:151–8.

82. Vukelic S, Griendling KK. Angiotensin ii, from vasoconstrictor to growth factor: A paradigm shift. Circ Res. 2014;114(5):754–7.

83. Kono R, Okuno Y, Nakamura M, Inada K, Tokuda A. Peach (Prunus persica) extract inhibits angiotensin II-induced signal transduction in vascular smooth muscle cells. Food Chem. Elsevier Ltd; 2013;139(1-4):371–6.

84. Lau FC, Shukitt-Hale B, Joseph JA. The beneficial effects of fruit polyphenols on brain aging. Neurobiol Aging. 2005;26(1):128–32.

85. Macready AL, Kennedy OB, Ellis JA, Williams CM, Spencer JPE, Butler LT. Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. Genes Nutr. 2009;4(4):227–42.

86. Mesías M, Navarro M, Gökmen V, Morales FJ. Antiglycative effect of fruit and vegetable seed extracts: Inhibition of AGE formation and carbonyl-trapping abilities. J Sci Food Agric. 2013;93(8):2037–44.

87. Szwajgier D, Borowiec K. Screening for cholinesterase inhibitors in selected fruits and vegetables. Electron J Polish Agric Univ. 2012;15(2).

88. Chatonnet A, Lockridgetl O. Comparison of butyrylcholinesterase and acetylcholinesterase. Biochem J. 1989;260:625–34.

89. Vizzotto M, Porter W, Byrne D, Cisneros-Zevallos L. Polyphenols of selected peach and plum genotypes reduce cell viability and inhibit proliferation of breast cancer cells while not affecting normal cells. Food Chem. Elsevier Ltd; 2014;164:363–70.

90. Noratto G, Porter W, Byrne D, Cisneros-Zevallos L. Polyphenolics from peach (Prunus persica var. Rich Lady) inhibit tumor growth and metastasis of MDA-MB-435 breast cancer cells in vivo. J Nutr Biochem. Elsevier Inc.; 2014;25(7):796–800.

91. Raturi R, Singh H, Bahuguna P, Sati SC, Badoni PP. Antibacterial and antioxidant activity of methanolic extract of bark of Prunus persica. 2011;(1):312–4.

92. Kumar DR, Kumar MA, Naidu PB. Evaluation of Anthelmintic Activity of Prunus persica (L.). 2015;4(05):716–21.

93. Silva LR, Queiroz M. Bioactive compounds of red grapes from Dão region (Portugal): Evaluation of phenolic and organic profile. Asian Pac J Trop Biomed. 2016;6(4):315–21.

94. Nunes AR, Alves MG, Tomás GD, Conde VR, Cristóvão AC, Moreira PI, et al. Daily consumption of white tea (Camellia sinensis (L.)) improves the cerebral cortex metabolic and oxidative profile in prediabetic Wistar rats. Br J Nutr. 2015;113(5):832–42.

95. Silva LR, Azevedo J, Pereira MJ, Valentão P, Andrade PB. Chemical assessment and antioxidant capacity of pepper (Capsicum annuum L.) seeds. Food Chem Toxicol. 2013;53:240–8.

96. Oliveira AP, Valentão P, Pereira JA, Silva BM, Tavares F, Andrade PB. Ficus carica L.: Metabolic and biological screening. Food Chem Toxicol. Elsevier Ltd; 2009;47(11):2841–6.

97. Chisté RC, Freitas M, Mercadante AZ, Fernandes E. Carotenoids are Effective Inhibitors of in vitro Hemolysis of Human Erythrocytes, as Determined by a Practical and Optimized Cellular Antioxidant Assay. J Food Sci. 2014;79(9):H1841–7.

98. Carvalho M, Ferreira PJ, Mendes VS, Silva R, Pereira JA, Jerónimo C, et al. Human

cancer cell antiproliferative and antioxidant activities of Juglans regia L. Food Chem Toxicol. Elsevier Ltd; 2010;48(1):441–7.

99. Tomás-Barberán F a, Gil MI, Cremin P, Waterhouse a L, Hess-Pierce B, Kader a a. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J Agric Food Chem. 2001;49(10):4748–60.

100. Mokrani A, Krisa S, Cluzet S, Da Costa G, Temsamani H, Renouf E, et al. Phenolic contents and bioactive potential of peach fruit extracts. Food Chem. Elsevier Ltd; 2016;202:212–20.

101. Bayram B, Ozcelik B, Schultheiss G, Frank J, Rimbach G. A validated method for the determination of selected phenolics in olive oil using high-performance liquid chromatography with coulometric electrochemical detection and a fused-core column. Food Chem. 2013;138(2-3):1663–9.

102. Martínez-Huélamo M, Tulipani S, Jáuregui O, Valderas-Martinez P, Vallverdú-Queralt A, Estruch R, et al. Sensitive and rapid UHPLC-MS/MS for the analysis of tomato Phenolics in human biological samples. Molecules. 2015;20(11):20409–25.

103. Gómez-Caravaca AM, Verardo V, Toselli M, Segura-carretero A, Fernández-Gutiérrez A, Caboni MF. Determination of the Major Phenolic Compounds in Pomegranate Juices by HPLC − DAD − ESI-MS. J Agric Food Chem. 2013;61:5328–37.

104. Huck CW, Buchmeiser MR, Bonn GK. Fast analysis of flavonoids in plant extracts by liquid chromatography – ultraviolet absorbance detection on poly (carboxylic acid) - coated silica and electrospray ionization tandem mass spectrometric detection. J Chromatogr A. 2002;943(2001):33–8.

105. Wang D, Lu J, Miao A, Xie Z, Yang D. HPLC-DAD-ESI-MS/MS analysis of polyphenols and purine alkaloids in leaves of 22 tea cultivars in China. J Food Compos Anal. 2008;21(5):361–9.

106. Liu J, Guo Y, Zhang J, Qi Y, Jia X, Gao G, et al. Systematic chemical analysis of flavonoids in the Nelumbinis stamen. Phytomedicine [Internet]. Elsevier GmbH.; 2014;21(13):1753–8. Available from: http://dx.doi.org/10.1016/j.phymed.2014.09.003

107. Liu AH, Li L, Xu M, Lin YH, Guo HZ, Guo DA. Simultaneous quantification of six major phenolic acids in the roots of Salvia miltiorrhiza and four related traditional Chinese medicinal preparations by HPLC-DAD method. J Pharm Biomed Anal. 2006;41(1):48–56.

108. Pellati F, Benvenuti S, Magro L, Melegari M, Soragni F. Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. J Pharm Biomed Anal. 2004;35(2):289–301.

109. Castañeda-Ovando A, Pacheco-Hernández MDL, Páez-Hernández ME, Rodríguez J a., Galán-Vidal CA. Chemical studies of anthocyanins: A review. Food Chem [Internet]. Elsevier Ltd; 2009;113(4):859–71. Available from:

http://dx.doi.org/10.1016/j.foodchem.2008.09.001

110. Zhao P, Duan L, Guo L, Dou LL, Dong X, Zhou P, et al. Chemical and biological comparison of the fruit extracts of Citrus wilsonii Tanaka and Citrus medica L. Food Chem. Elsevier Ltd; 2014;173:54–60.

111. Wang BN, Cao W, Gao H, Fan MT, Zheng JB. Simultaneous Determination of Six Phenolic Compounds in Jujube by LC-ECD. Chromatographia. 2010;71(7-8):703–7.

112. Tóth G, Alberti Á, Sólyomváry A, Barabás C, Boldizsár I, Noszál B. Phenolic profiling of various olive bark-types and leaves: HPLC-ESI/MS study. Ind Crops Prod. Elsevier B.V.; 2015;67:432–8.

113. Abu a H, Uchendu CN. Antispermatogenic effects of aqueous ethanolic extract of Hymenocardia acida stem bark in wistar rats. J Med Plant Res. 2010;4(23):2495–502.

114. Liu H, Cao J, Jiang W. Changes in phenolics and antioxidant property of peach fruit during ripening and responses to 1-methylcyclopropene. Postharvest Biol Technol. Elsevier B.V.; 2015;108:111–8.

115. Mandel S, Youdim MBH. Catechin Polyphenols: Neurodegeneration and Neuroprotection in Neurodegenerative Diseases. Free Radic Biol Med. 2004;37(3):304–17.

116. Ivanovic J, Tadic V, Dimitrijevic S, Stamenic M, Petrovic S, Zizovic I. Antioxidant properties of the anthocyanin-containing ultrasonic extract from blackberry cultivar “Cacanska Bestrna.” Ind Crops Prod. Elsevier B.V.; 2014;53:274–81.

117. Barzegar H, Ghavidel RA, Einafshar S. Ultrasonic Extraction of Antioxidants from Different Species of Wild Hawthorn Leaves. Int J Rev Life Sci. 2015;5(9):1709–15.

118. Gulfishan, Akhtar N. Prunus Domestica, Prunus persica and Prunus avium extracts: determination of radical scavenging activities and development of natural antioxidant emulsions. Afr J Tradit Complement AlternMed. 2014;11(6):47–52.

119. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). Food Chem. 2008;111(4):925–9.

120. Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol. 2003;41(6):753–8.

121. Kim K, Yu S, Yook H. Antioxidant and Whitening Activities of Various Cultivars of Korean Unripe Peaches (Prunus persica L. Batsch). J Korean Soc Food Sci Nutr. 2012;41(2):156–60.

122. Lapshina EA, Zamaraeva M, Cheshchevik VT, Olchowik-Grabarek E, Sekowski S, Zukowska I, et al. Cranberry flavonoids prevent toxic rat liver mitochondrial damage in vivo and scavenge free radicals in vitro. Cell Biochem Funct. 2015;33(4):202–10.

123. Bhattacharjee M, Kamal R, Roy L. Evaluation of Free Radical Scavenging Activity of “Prunus Avium.” Int J Pharm Sci Rev Res. 2016;38(2):1-5.

124. Yokozawa T, Chen CP, Tanaka T. Direct scavenging of nitric oxide by traditional crude drugs. Phytomedicine. Urban & Fischer Verlag; 2000;6(6):453–63.

125. Zhang WT, Gao K, Liu JW, Zhao HH, Wang J, Li YB, et al. A Review of the Pharmacological Mechanism of Traditional Chinese Medicine in the Intervention of Coronary Heart Disease and Stroke. African J Tradit Complement Altern Med. 2013;10(6):532–7.

126. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539–53.

127. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care [Internet]. 2004;27(Supplement 1):S5–10. Available from: http://care.diabetesjournals.org/cgi/doi/10.2337/diacare.27.2007.S5

128. Nunes AR, Alves MG, Moreira PI, Oliveira PF, Silva BM. Can Tea Consumption be a Safe and Effective Therapy Against Diabetes Mellitus-Induced Neurodegeneration? Curr Neuropharmacol. 2014;12(6):475–89.

129. Sakulnarmrat K, Srzednicki G, Konczak I. Composition and inhibitory activities towards digestive enzymes of polyphenolic-rich fractions of Davidson’s plum and quandong. LWT - Food Sci Technol. 2014;57(1):366–75.

130. Patil A, Nirmal S, Pattan S, Tambe V, Tare M. Antidiabetic effect of polyherbal combinations in STZ induced diabetes involve inhibition of α-amylase and α-glucosidase with amelioration of lipid profile. Phytopharmacology. 2012;2(1):46–57.

131. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. J Nutr Sci Vitaminol (Tokyo). 2006;52(2):149–53.

132. Ma YY, Zhao DG, Zhou AY, Zhang Y, Du Z, Zhang K. a-Glucosidase Inhibition and Antihyperglycemic Activity of Phenolics from the Flowers of Edgeworthia gardneri. J Agric Food Chem. 2015;63(37):8162–9.

133. Costa RM, Magalhães AS, Pereira JA, Andrade PB, Valentão P, Carvalho M, et al. Evaluation of free radical-scavenging and antihemolytic activities of quince (Cydonia oblonga) leaf: A comparative study with green tea (Camellia sinensis). Food Chem Toxicol. Elsevier Ltd; 2009;47(4):860–5.

134. Barros L, Ferreira MJ, Queir??s B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, ??-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem. 2007;103(2):413–9.

135. Rocha S, Costa E, Rocha-Pereira P, Ferreira F, Cleto E, Barbot J, et al. Erythrocyte membrane protein destabilization versus clinical outcome in 160 Portuguese Hereditary Spherocytosis patients. Br J Haematol. 2010 Jun;149(5):785–94.

136. Mendes L, De Freitas V, Baptista P, Carvalho M. Comparative antihemolytic and radical

scavenging activities of strawberry tree (Arbutus unedo L.) leaf and fruit. Food Chem Toxicol. 2011;49(9):2285–91.

137. Magalhães AS, Silva BM, Pereira JA, Andrade PB, Valentão P, Carvalho M. Protective effect of quince (Cydonia oblonga Miller) fruit against oxidative hemolysis of human erythrocytes. Food Chem Toxicol. Elsevier Ltd; 2009;47(6):1372–7.

138. Peyrat-Maillard MN, Cuvelier ME, Berset C. Antioxidant activity of phenolic compounds in 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidation: Synergistic and antagonistic effects. J Am Oil Chem Soc. 2003;80(10):1007–12.

139. Natella F, Nardini M, Di Felice M, Scaccini C. Benzoic and cinnamic acid derivatives as antioxidants: Structure- activity relation. J Agric Food Chem. 1999;47(4):1453–9.

140. Sulaiman A, Hussain S. Concentration and time dependent cytoprotective effects of anthocyanins against oxidative hemolysis induced by water and lipid soluble free radical initiators: an in vitro study. Oxid Antioxid Med Sci. 2012;1(2):1.

141. Chisté RC, Freitas M, Mercadante AZ, Fernandes E. Carotenoids inhibit lipid peroxidation and hemoglobin oxidation, but not the depletion of glutathione induced by ROS in human erythrocytes. Life Sci. 2014;99(1-2):52–60.

142. Mariutti LRB, Rodrigues E, Chisté RC, Fernandes E, Mercadante AZ. The Amazonian fruit Byrsonima crassifolia effectively scavenges reactive oxygen and nitrogen species and protects human erythrocytes against oxidative damage. Food Res Int. Elsevier Ltd; 2014;64:618–25.

143. Kanias T, Acker JP. Biopreservation of red blood cells - The struggle with hemoglobin oxidation. FEBS J. 2010;277(2):343–56.