Friedrich Schiller University Jena
Faculty of Biological Sciences
Institute of Nutritional Sciences
Bioactive Plant Products Research Group

In vitro investigations on biological activities and bioaccessibility of rosehip carotenoids

Dissertation

To fulfil the Requirements for the Degree of
„*doctor rerum naturalium*“ (Dr. rer. nat.)

Submitted to the Council of the Faculty of Biological Sciences of the Friedrich Schiller University Jena

By: Ahlam Al-Yafeai

born on 25th December 1979 in Ibb, Yemen

Reviewer

1. PD Dr. habil. Volker Böhm

Friedrich Schiller University Jena
Institute of Nutritional Sciences
Bioactive Plant Products Research Group
Dornburger Str. 25
07743 Jena

2. Prof. Dr. Anna Kipp

Friedrich Schiller University Jena
Molecular Nutritional Physiology
Dornburger Str. 24
07743 Jena

3. Prof. Dr. Marcus A. Glomb

Martin-Luther-Universität Halle-Wittenberg
Institut für Chemie - Lebensmittelchemie
Martin-Luther-Universität Halle-Wittenberg
Kurt-Mothes-Str. 2
06120 Halle (Saale)

Date of defense: 1th of April 2019

Table of contents

Abbreviation list

$^1O_2$:	Singlet molecular oxygen
AA:	Ascorbic acid
AOC:	Antioxidants capacity
DAGs:	Diacylglycerols
DHA:	Dehydroascorbic acid
DM:	Degree of methylation
FFAs:	Free fatty acids
HG:	Homogalacturonan
LDL:	Low-density lipoprotein
MAGs:	Monoacylglycerols
PE:	Phytoene
PF:	Phytofluene
PG:	Endopolygalacturonase
PL:	Pectinlyase
PME:	Pectinmethylesterase
R.C.Ja:	*Rosa canina* jam
R.C.Pr:	*Rosa canina* powder
R.C.Pu:	*Rosa canina* puree
R.C.R:	*Rosa canina* raw
RGI:	Rhamnogalacturonan type I
RGII:	Rhamnogalacturonan type II
RNS:	Reactive nitrogen species
ROO$^{\bullet}$:	Peroxyl radicals
ROS:	Reactive oxygen species
TAGs:	Triacylglycerols
TP:	Total phenolic compounds

Register of figures and tables

1 Introduction

1.1 Rosehips

Family: Rosaceae

Genus: *Rosa*

Section used in the present thesis work: *rugosa* Thunb. and *canina*

The genus Rosa contains over 100 species that are widely distributed mostly in Europe, Asia, the Middle East and North America, these species differ in their appearance and chemical composition (Nilsson, 1997). *Rosa rugosa* Thunb. and *Rosa canina* L. are the most important ones in the food industry in Europe. Rugosa hips resemble tomatoes with a 2-3 cm diameter and often a shorter height than their diameter, whereas canina hips are smaller and the mature hips are oval with a diameter of 1.5-2 cm (Al-Yafeai *et al.*, 2018; Andersson *et al*., 2011& Henker, 2000). Rosehips have a rather long ripening period and usually stay attached on the bushes long after the first night frost, harvesting only once per year during autumn. In late summer and early autumn, the plants often bear fruits and flowers at the same time.

During ripening, the hips start to change colour at sunny side from green via yellow-orange to dark red and sometimes even black, depending on the pattern of pigments such as carotenoids, flavonoids, or anthocyanins. In recent years, there has been a general increase in the interest in using plants as an alternative source of raw materials for pharmaceutical, food and cosmetic industries. Numerous studies confirm that many plant components are - in addition to be nutritionally valuable - also important in the prevention of civilization diseases (Youdim *et al.*, 2000; Zafra-Stone *et al.*, 2007). A number of epidemiological studies have shown that a high intake of fruit and vegetables is correlated with positive effects on human health, and decreases

the incidence of diseases such as cardiovascular diseases, cataracts and different cancers and other chronic diseases (Lampe, 1999; Gandini *et al.*, 2000; Liu *et al.*, 2000; Joshipura *et al.*, 2001 & Liu *et al.*, 2001). For this reason, the food industry now focuses on developing new products with potential impact on public health and the nutritional status of the population.

Recently, rosehips have been increasingly studied for their preventive properties. However, the available data advocating the medicinal importance of rose hip formulations are sparse and disorganized (Seema, 2012). Although *R. rugosa* is renowned for producing the most abundant and best tasting hips, most food products are based on the hips of *R. canina* although they are small compared with the rugosa hips (Barros *et al.*, 2011). Several rosehip extracts have been released to the market as nutritive aids, health supplements, cosmetics and herbal remedies for the treatment of diseases, including cold, inflammation, osteoarthritis, rheumatoid arthritis and chronic pain in China (Guo *et al.*, 2011). Moreover, some rosehips are used to prepare 'Nypon soppa', the traditional Swedish fruit soup. *R. eglanteria* hips infusion is popular in Europe as herbal tea. In the Tokat region of Anatolia, Turkey, rosehips are consumed as jam and fruit juice (Güne, 2010).

Later, cosmetology research has proven the effect of rosehips oil in lowering skin pigmentation, reducing scars and stretches, acne management, rehydrating skin and rendering it supple and delaying wrinkling. Even the skin specialists are recommending the use of rosehips oil as skin vitalizing agent. Daels-Rakotoarison *et al.* (2002) & Halvorsen *et al.* (2002) reported that the antioxidant activity of rosehips extracts was higher compared with other fruit and berries. Several *in vivo* studies in animals revealed that rosehips extracts have a strong effect on body fat, plasma and biliary lipids (Gonzales *et al.*, 1997& Ninomiya *et al.*, 2007). In the same vein, the

investigations on anti-inflammatory activity have shown that lipophilic extracts of rosehips have a higher activity than hydrophilic extracts (Deliorman *et al.*, 2007 & Wenzig *et al.*, 2007).

Although clinical studies to evaluate the effects of rosehips on humans suffering from osteoarthritis, rheumatoid and arthritis have shown positive results, studies are not yet adequate and need further proof (Christensen *et al.*, 2007; Rossnagel *et al.*, 2007& Chrubasik *et al.*, 2008). In addition, rosehips extracts have anti-microbial effects on different bacteria (Kumarasamy *et al.*, 2002), anti-mutagenic effects on Salmonella typhimurium and anti-cancerogenic effects in different *in vitro* studies on cancer cells (Karakaya & Kavas 1999 & Olsson *et al.*, 2004). In the last few years, much more information on rosehips has become available, the physiological functions of rosehip fruits may be partly attributed to their abundance of bioactive compounds. Previous studies reported that rosehips contained higher amounts of various bioactive compounds than several other fruits and berries such as: flavonoids, tannins, carotenoids, phytoene, fatty acids and vitamins (particularly vitamins C, E and provitamin A) (Böhm *et al.*, 2003 & Al-Yafeai *et al.*, 2018). Furthermore, rose hips extract contained high contents of pectin (Al-Yafeai & Böhm 2018).

1.2 Carotenoids

Carotenoid pigments, responsible for the colour of a wide variety of foods, are mainly C_{40} lipophilic isoprenoids having a polyene skeleton, consisting of a long conjugated double-bond system. The basic skeleton may be modified in many ways, including cyclization, hydrogenation, dehydrogenation, introduction of oxygen functions, rearrangement, chain shortening, or combinations thereof, resulting in a multitude of carotenoids structures. Almost all carotenoids absorb light in the 400-500

nm range (Britton *et al.*, 1995 & 2004). As the number of conjugated double bonds increases, the absorbance wavelength of the chromophore also increases, therefore, its colour moves to longer wavelengths (bathochromic shift).

Carotenoids are synthesized in all photosynthetic organisms (bacteria, algae and plants) as well as in some nonphotosynthetic bacteria and fungi. Although carotenoids are necessary to maintain normal health and behavior of animals, carotenoids are not synthesized by animals and so those found in animals are directly accumulated from food (Agarwal *et al.*, 2012). Despite approximately 750 carotenoids have been reported (Britton *et al*., 2004), only those with an unsubstituted β-ring with an 11-carbon polyene chain have provitamin A activity. This structural requirement is satisfied by around 60 carotenoids, which are subsequently transformed into vitamin A (Rodriguez-Amaya, 2001). Free and esterified carotenoid compounds can be found in the natural world and the free carotenoids can be divided into hydrocarbon carotenoids (known as carotenes), examples of the carotenes are α-, β-, γ-, ζ- carotene and lycopene. Oxygenated derivatives are called xanthophylls, which are esterified with fatty acids in matured fruits and vegetables, generating xanthophyll esters. Common oxygen substituents are the hydroxy (as in β-cryptoxanthin), keto (as in canthaxanthin), epoxy (as in violaxanthin) and aldehyde (as in β-citraurin) groups. Carotenoids can be acyclic (e.g., lycopene), monocyclic (e.g., γ-carotene) or dicyclic (e.g., α- and β-carotene) (Britton *et al.*, 2004 & Krinsky & Johnson, 2005).

In nature, carotenoids mostly occur as *(all-E)*-isomers in plants. However, contents of *(Z)*-isomers may increase due to the isomerization of the *(all-E)*-isomers of carotenoids during food processing. Carotenoids are very unstable because they are highly unsaturated, being easily deteriorated by oxidative processes. Heat, light, drying

and structural differences are the prominent factors that affect the isomerisation of carotenoids in foods (Schieber & Carle, 2005).

1.2.1 Biological activity of carotenoids

Carotenoids have been important in some diseases treatment and prevention, with antitumor properties and protection against free radicals, lipid peroxidation, oxidative damage to LDL-cholesterol, oxidation of essential polyunsaturated fatty acids and the UV light protection effects on cell membranes and tissues (Hu *et al.*, 2006). Moreover, xanthophylls showed antioxidant power 10 times greater than β-carotene and 500 times higher than vitamin E (Lopez *et al.*, 2004).

The recent interest in specific carotenoids for different health-food purposes implicate that an increased knowledge of specific carotenoids is of high importance for health issues e.g. lycopene, for which rose hips have been proposed as a source of raw material (Böhm *et al.*, 2003). Many of the carotenoids found in rosehips, such as zeaxanthin, lutein, lycopene and β-carotene, have been shown to have health beneficial effects (Bohn, 2008 & Andersson *et al.*, 2011). Clinical and epidemiological studies have confirmed that diets rich in lycopene are associated with reduced risk of developing prostate, lung and ovary cancers and a lower incidence of chronic degenerative diseases and cardiovascular diseases (Rao, 2002 & Cramer *et al.*, 2001). Lutein and zeaxanthin are stored in our body in the retina and lens of eyes (Thane & Reddy 1997).

The major provitamin A carotenoid in the Western diet is (β)-carotene but also (α)-carotene, (β)- and (α)-cryptoxanthin contribute to vitamin A supply and may prevent vitamin A deficiency. Vitamin A is an essential nutrient that maintains human health by controlling vision, immune system, reproduction, development and cell

growth (D'Ambrosio *et al.*, 2011). During embryonic development, vitamin A is vital as both vitamin A excess and deficiency cause embryonic defects (Abu-Abed *et al.*, 2001). A mammalian embryonic development relies on the maternal circulating retinoids and the essential nutrient vitamin A is obtained only from the diet, maternal vitamin A intake and status directly affect fetal development. β-Carotene is currently used as dietary supplement due to its health-promoting effects (Bohn, 2008 & Andersson *et al.*, 2011).

1.2.2 Carotenoids as antioxidants

The antioxidant properties of carotenoids are associated with their radical scavenging properties against two reactive oxygen species (ROS): singlet molecular oxygen ($^1O_2$) and peroxyl radicals ($ROO^{\bullet}$) (Everett *et al.*, 1996). Furthermore, carotenoids are effective deactivators of electronically excited sensitizer molecules which are involved in the generation of radicals and singlet oxygen (Young & Lowe, 2001). Quenching constants for $^1O_2$ have been determined for a range of carotenoids in solution and reveal a clear relationship between structure of the carotenoid and the ability to quench this particular ROS by energy transfer (Baltschun *et al.*, 1997). Carotenoids may interact with free radicals in three main ways, namely electron-transfer (ET) (Eq. [1]), hydrogen atom transfer (HAT) (Eq. [2]) and addition of a radical species (Eq. [3]) (Britton *et al.*, 1995).

$$ROO^{\bullet} + CAR \longrightarrow ROO^{-} + CAR^{\bullet} \quad [1]$$

$$ROO^{\bullet} + CAR \longrightarrow ROOH + CAR^{\bullet} \quad [2]$$

$$ROO^{\bullet} + CAR \longrightarrow (ROO\text{-}CAR)^{\bullet} \quad [3]$$

The interaction of carotenoids with $^1O_2$ depends largely on physical quenching which involves direct energy transfer between both molecules. The energy of singlet molecular oxygen is transferred to the carotenoid molecule to yield ground state oxygen and a triplet excited carotene. Instead of further chemical reactions, the carotenoid returns to ground state, dissipating its energy by interaction with the surrounding solvent. In contrast to physical quenching, chemical reactions between the excited oxygen and carotenoids are of minor importance, contributing less than 0.05% to the total quenching rate. Since the carotenoids remain intact during physical quenching of $^1O_2$ or excited sensitizers, they can be reused several fold in such quenching cycles. The efficacy of carotenoids for physical quenching is related to the number of conjugated double bonds present in the molecule, which determines their lowest triplet energy level. The most efficient carotenoid is the acyclic carotenoid lycopene, which contributes up to 30% to total carotenoids in humans (Baltschun *et al.*, 1997).

Beside singlet oxygen, peroxyl radicals are generated in the process of lipid peroxidation, and scavenging of this species interrupts the reaction sequence which finally leads to damage in lipophilic compartments. Carotenoids, as highly lipophilic molecules, are expected to be particularly effective scavengers of ROS within the hydrophobic portions of cell membranes and lipoproteins, their major transporters, reducing the possibility of oxidation of membrane structures and the overall risk of morbidity (Agarwal *et al.*, 2012).

Other colourless carotenoids being expected to find in rosehips are phytoene (PE) and phytofluene (PF). PE and PF are the common carotenoid precursors to the downstream carotenoid products found in plants. Therefore, PE and PF are conceivably present to some degree in a large array of carotenoid-containing foods (Britton *et al.*, 1995).

1.3 Vitamin E

Vitamin E ($C_{29}H_{50}O_2$) is a lipophilic antioxidant, the term vitamin E covers eight fat-soluble compounds (α-, β-, γ-, δ-tocopherol and -tocotrienol). The basic structure of vitamin E comprises a hydrophobic polyprenyl side chain with a polar chromanol ring. Tocochromanols with a fully saturated side chain are called tocopherols and those with an unsaturated at positions 3', 7' and 11' of the side chain side are tocotrienols. The specific tocopherols and tocotrienols differ by number and positions of the methyl groups in the 6-chromanol ring, resulting in the α-, β-, γ- and δ-isomers (Munné-Bosch & Alegre 2002).

Tocopherols occur in mammalians, photosynthetic bacteria, fungi, algae and plants, but only photosynthetic organisms are able to synthesize them. The biosynthesis is initiated in the plant's cytoplasm, but, except for this first step, its biosynthesis takes place in the plastids (Grusak & Dellapenna 1999 & Munné-Bosch & Alegre 2002). The content, composition and presence of tocopherols vary widely in different plant tissues. They can be found in seeds, fruits, roots and tubers and are usually present in the green parts of higher plants (Mene-Saffrane & Dellapenn 2010 Horvath *et al.*, 2006).

Vitamin E was first discovered as a necessary nutritional factor in pregnant rats for foetus viability (Evans & Bishop 1922). Despite vitamin E's importance in the human diet, dietary studies showed that the recommended daily allowance is often not met, so, improving its quantity and composition has become a target in crop breeding (Péter *et al.*, 2016). In general, vitamin E is found in high concentrations in vegetable oils such as almond, safflower or canola oil or in other high-fat sources such as nuts, seeds or grains (Dellapenna, 2005). Food-grade canola oil, or 00-type rapeseed oil, has a high-quality nutritional composition similar to that of olive oil, and tocopherols are one of the main nutritionally relevant constituents (Péter *et al.*, 2016).

1.3.1 Health effects of vitamin E

α-Tocopherol is the most common form of vitamin E in human tissues and has long been considered as a protective factor, preventing inflammatory and degenerative processes in the liver during the exposure to a range of xenobiotics, environmental pollutants and dietary factors (Aboul-Soud *et al.*, 2011). Furthermore, vitamin E is necessary in animal cells for different antioxidant functions, especially in cell membranes and plasma lipoproteins. It participates in preventing the proliferation of oxidative chain reactions, and it plays possible roles in atherosclerosis and cancer prevention (Lopaczynski & Zeisel 2001 & Bender & Mayes 2003). A number of epidemiological studies have shown that a high intake of vitamin E is correlated with positive effects on human health, and decreases the incidence of diseases such as cardiovascular diseases and different types of cancers (Albanes *et al.*, 1995) as well as Alzheimer's disease (Morris *et al.*, 2005).

1.3.2 Vitamin E as antioxidant

Vitamin E (α-tocopherol) is an efficient lipid soluble antioxidant that functions as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles, including low-density lipoprotein (LDL). It functions to intercept lipid peroxyl radicals ($LOO^{\bullet}$) and to terminate the lipid peroxidation chain reactions. It interacts with polyunsaturated acyl groups and protects polyunsaturated fatty acids from lipid peroxidation by scavenging lipid peroxy radicals and quenching ROS produced e.g., by photosystem II and during membrane lipid peroxidation (Brigelius-Flohe & Traber 1999 & Krieger-Liszkay *et al.*, 2008). During this process, tocopherols donate their phenolic hydrogen to lipid-free radicals, thus neutralizing the radical, terminating the autocatalytic lipid peroxidation processes and protecting cell

membranes. The resultant tocopheroxyl radical is relatively stable and under normal circumstances insufficiently reactive to initiate lipid peroxidation itself, which is an essential criterion of a good antioxidant (Kamal-Eldin & Appelqvist 1996 & Liebler, 1993).

$LOO^{\bullet}$ + α-tocopherol–OH /LOOH + α-tocopherol–$O^{\bullet}$

Furthermore, tocopherols are able to deactivate $^1O_2$, which oxidizes, amongst others, membrane lipids, proteins, amino acids, nucleic acids, nucleotides and carbohydrates (Halliwell & Gutteridge 2015 & Straight & Spikes 1985). The chemical reaction of tocopherols with $^1O_2$ results in the corresponding tocopherol quinones (and other derivatives), some of which have been shown to be potent antioxidants (Lass & Sohal 1998 & Siegel *et al.*, 1997).

1.4 Vitamin C (Ascorbic acid)

Vitamin C (Ascorbic acid, AA) is the most powerful water-soluble antioxidant in human blood plasma and acts as a regenerator for vitamin E in lipid systems. AA is an odorless, white solid, having the chemical formula $C_6H_8O_6$ (Groff *et al.*, 1995), being ascorbic acid and its first oxidation product, dehydroascorbic acid (DHA) (Gokmen *et al.*, 2000). Therefore, the total vitamin C contents in fruit are the sum of these two biologically active compounds, AA and DHA (Nováková *et al.*, 2008; Valente *et al.*, 2014). Most physiological roles of AA are linked to its capability to be a reducing agent in a biochemical reaction (Tsaniklidis *et al.*, 2014). AA significantly decreases the adverse effects of the ROS and RNS which cause oxidative damage to lipids, DNA and proteins (Padayatty *et al.*, 2003).

In addition to the antioxidant properties of AA, it also has major roles in hormone signaling and plant development, cell cycle, cell expansion, as a part of the

cellular redox system and as a cofactor for several important enzymes (Davey et al., 2000 & Smirnoff *et al.*, 2001). An enhanced fruit AA pool has also been suggested to be associated with improved postharvest fruit quality in hard fruit species, such as pear and apple (Franck et al., 2003 & Davey et al., 2007). AA contributing to the antioxidant capacity of plant tissues, especially during different stressful environments through detoxify ROS and free radicals such as $^1O_2$, hydroxyl radical (OH-), superoxide (O_2-) and hydrogen peroxide (H_2O_2), produced during metabolic processes of photosynthesis (Smirnoff, 2011).

Most plants and animals have the ability to synthesize vitamin C, only mammals including humans are unable to synthesize vitamin C. Therefore humans depend on exogenous sources of vitamin C including vegetables, fruits as well as food supplements and pharmaceutical preparations (Okiei *et al.*, 2009). One of the most important features in rosehips is the high content of vitamin C. (Erenturk *et al.*, 2005). Generally, rosehips are considered to be the most abundant natural source of vitamin C, the contents ranged between 200 and 2800 mg/100 g (Uggla *et al.*, 2003). Thus, the medicinal value of rosehips depends largely on the vitamin C contents value of rosehips. Moreover, vitamin C terminates the lipid peroxidation chain reaction by donating an electron to the lipid radical and rapidly changes to the ascorbate radical.

1.5 Bioaccessibility and Bioavailability

As the potential health benefits of bioactive compounds such as carotenoids and vitamins become more and more clear, there has been a growing interest in determination of bioaccessibility and bioavailability of these compounds from plant foods. Bioaccessibility is defined as the fraction of a compound that releases from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption

(Reboul *et al.*, 2006). On the other hand, the term "bioavailability" is usually defined as the fraction of a given compound or its metabolite that reaches the systemic circulation (Holst & Williamson 2008).

However, human intervention studies to assess intestinal absorption are expensive, often invasive and of long duration. Static *in vitro* models based on human physiology were developed as simple, inexpensive and reproducible tools to predict the bioaccessibility of different food components, allowing large numbers of samples to be tested. Although one of the most relevant factors limiting the bioaccessibility of carotenoids at the pre-absorptive stages is the food matrix, Minekus *et al.*, (2014) aimed to find a standardized processing for an *in vitro* digestion model.

Simulated digestion methods typically include the oral, gastric and small intestinal phases, and occasionally large intestinal fermentation. These methods try to mimic physiological conditions *in vivo*, taking into account the presence of digestive enzymes and their concentrations, pH, digestion time and salt concentrations, among other factors. In recent years, there has been an increasing amount of literature on the effects of carotenoids and vitamin E from different natural sources on health.

Knowledge of lipid digestion is important for understanding of absorption of lipophilic food ingredients. Dietary lipids enhanced secretion of bile salts and led to a stimulation of the activity of pancreatic lipase, which in turn increased micellarization capacity (Furr & Clark 1997). Pancreatic lipase facilitated the transfer of carotenoids from emulsified lipid droplets toward mixed micelles (Borel *et al.*, 1998). Therefore, lipid digestion can be divided into two steps: (i) the hydrolysis and (ii) the micellarization. Hydrolysis of dietary triacylglycerols (TAGs) into diacylglycerols (DAGs), monoacylglycerols (MAGs) and free fatty acids (FFAs) is essential for their absorption by enterocytes and their incorporation into micelles (Mcclements & Decker

2009). The micellarization is a process to form molecular aggregates of 3-10 nm (micelles) through the action of bile salts on lipid particles (Johnson & Gerwin 2001). The micelles are composed of MAGs and FFAs, bile salts, phospholipids and lipid-soluble compounds (Yonekur & Nagao 2007).

1.5.1 Pectin and Bioaccessibility

Although soluble fiber consumption has widely recognized health benefits, the impact of pectin and other dietary fibers on the bioavailability and metabolism of other nutrients, including lipids, is still under investigation. Some *in vitro* studies showed for pectin a potential to impact lipid digestion processes (Zhang *et al.*, 2015) which can affect the carotenoid's bioaccessibility. Pectic polysaccharides, generally referred to as "pectin," are one of the most structurally complex and fascinating naturally occurring plant macromolecules.

The molecular structure of both extracted and plant-located pectin is very diverse, varying with the physiological state of the plant and even between tissues in the plant (Bagherian *et al.*, 2011, Christiaens *et al.*, 2011 & Guo *et al.*, 2014). The backbone of pectin is composed of segments of homogalacturonan (HG) and rhamnogalacturonan type I (RGI) and II (RGII) (Sila *et al.*, 2009), which are esterified with a varying degree of methylation (DM) (Wicker *et al.*, 2014). HG is the most abundant pectic polysaccharide accounting for about 65% of the total pectin content of plant tissues (Mohnen, 2008). This linear structural element of pectin is a homopolymer of up to 200 units of α (1→4)-linked D-galacturonic acid (GalA) (Thibault *et al.*, 1993 & Zhan *et al.*, 1998). RGI, representing approximately 20% to 35% of pectin (Albersheim *et al.*, 1996), RGII, making up approximately 10% of pectin, is the most conserved and complex structural element of pectin. On the other hand, Fructozym P6-

XL is a liquid, highly concentrated pectolytic enzyme mixture containing pectinmethylesterase (PME), pectinlyase (PL) and endopolygalacturonase (PG), and it is used to complete pectin degradation in juice for a good clarification and filterability.

2 Objectives

Although rosehips have more recently attracted attention because of their potential health benefits, there is little information about the change of antioxidants contents in different rosehip products as well as depending on the degree of ripeness, especially in rugosa hips. Furthermore, there is no study having investigated the bioaccessibility of carotenoids and vitamin E from rosehip raw materials as well as its products.

Hence, the following specific aims can be formulated for the present thesis:

Objective 1: The main objective of this doctoral thesis was to draw attention to *R. rugosa.* To this end, identification and quantification of carotenoids, vitamin E and vitamin C contents, as well as evaluation of antioxidants capacity in different products of canina hips and at different ripening times in rugosa hips was carried out.

Objective 2: Determination of the bioaccessibility of carotenoids and vitamin E in rosehips (*R. rugosa* and *R. canina*), canina products (powder, jam, and puree) as well as tomato paste.

Investigation of the effects of pectin, food processing and carotenoids properties on the bioaccessibility in selected samples of both rosehips and tomato paste.

3 Schedule of manuscripts

Manuscript: I

Characterization of carotenoids and vitamin E in *R. rugosa* and *R. canina*: Comparative analysis

Ahlam Al-Yafeai, Angelika Malarski and Volker Böhm

Food Chemistry 242 (2018) 435-442
http://dx.doi.org/10.1016/j.foodchem.2017.09.070
Accepted 13 September, 2017; Published 14 Septembers, 2017

Manuscript: II

In Vitro Bioaccessibility of Carotenoids and Vitamin E in Rosehip Products and Tomato Paste As Affected by Pectin Contents and Food Processing

Ahlam Al-Yafeai and Volker Böhm

Journal of Agricultural and Food Chemistry (2018) 66, 3801-3809
DOI: 10.1021/acs.jafc.7b05855
Accepted: 1 April, 2018; Published: 6 April, 2018

Manuscript: III

Bioactive compounds and antioxidant capacity of *R. rugosa* depending on degree of ripeness

Ahlam Al-Yafeai, Peter Bellstedt and Volker Böhm

Antioxidants (2018) 7, 134
DOI: 10.3390/antiox7100134
Accepted: 25 September, 2018; Published: 3 October, 2018

3.1 Manuscript: I

Characterization of carotenoids and vitamin E in *R. rugosa* and *R. canina*: Comparative analysis

Ahlam Al-Yafeai, Angelika Malarski and Volker Böhm

Food Chemistry 242 (2018) 435-442
http://dx.doi.org/10.1016/j.foodchem.2017.09.070
Accepted 13 September, 2017; Published 14 Septembers, 2017

ABSTRACT: The hips of Rosa species have gained more attention in recent years due to their high contents of antioxidant compounds. This study was designed to compare rosehips of the two roses species *Rosa rugosa* and *Rosa canina*, including different products, on carotenoid contents, including phytoene and phytofluene, as well as vitamin E. The investigation allowed the identification and quantification of types of *(Z)*-isomers of lycopene and rubixanthin in both rosehips and focused also on isomerisation of both carotenoids. The carotenoid identification and quantification were done using HPLC-DAD and LC-MS/MS. The statistical analysis revealed significant differences ($P < 0.05$) in carotenoid contents and ($P < 0.001$) in vitamin E contents between different rosehips species. The HPLC analysis showed that carotenoid contents varied between rosehips species. The isomerization of *(all-E)*-rubixanthin and *(all-E)*-lycopene using iodine-catalysed photoisomerisation showed that the (5′Z)-isomer gazaniaxanthin is the main *(Z)*-isomer of rubixanthin and the *(13Z)*-isomer is the main *(Z)*-isomer of lycopene.

Work share

Ahlam Al-Yafeai:	Identification and quantification of carotenoids and vitamin E in rosehip samples by HPLC, Identification and quantification of *(all-E)*- and *(Z)*-lycopene by HPLC, Fractionation and isolation of *(all-E)*-rubixanthin, Iodine isomerization of rubixanthin and lycopene isomers and identification by using HPLC and spectrophotometer, statistical analysis and preparation of the manuscript. Total share: 80%
Angelika Malarski:	LC-MS analysis of carotenoids Total share: 10%
Volker Böhm:	Correction of the manuscript Total share: 10%

3.2 Manuscript: II

In Vitro Bioaccessibility of Carotenoids and Vitamin E in Rosehip Products and Tomato Paste As Affected by Pectin Contents and Food Processing

Ahlam Al-Yafeai and Volker Böhm

Journal of Agricultural and Food Chemistry (2018) 66, 3801-3809
DOI: 10.1021/acs.jafc.7b05855

Accepted: 1 April, 2018 Published: 6 April, 2018

ABSTRACT: Limited bioavailability of antioxidants present in food from fruits and vegetables matrices is determined by their low bioaccessibility due to the physical and chemical interactions of the antioxidants with the indigestible polysaccharides of cell walls. Therefore, this in vitro investigation aimed to assess the bioaccessibility of carotenoids and vitamin E from rosehips as well as from tomato paste and to investigate several aspects of effects of pectin contents and food processing on bioaccessibility. Following the addition of the enzyme mixture Fructozym P6-XL, the bioaccessibility of carotenoids from rosehips as well as from tomato paste significantly increased. The average relative increase in bioaccessibility from rosehips was lower for *(all-E)*-βcarotene compared with *(all-E)*-lycopene and *(all-E)*-rubixanthin. In contrast, increases of bioaccessibility of α-tocopherol were comparable for rosehip samples and tomato paste.

Work share:

Ahlam Al-Yafeai:	Design of an *in vitro* model, quantification of carotenoids, vitamin E and pectin in rosehip products as well as tomato paste before and after simulated digestion model as well as after treated with Fructozym P6-XL by using HPLC, calculation and statistical analysis and preparation of the manuscript. Total share: 90%
Volker Böhm:	Correction of the manuscript Total share: 10%

3.3 Manuscript: III

Bioactive compounds and antioxidant capacity of *R. rugosa* depending on degree of ripeness

Ahlam Al-Yafeai, Peter Bellstedt and Volker Böhm

Antioxidants (2018) 7, 134
DOI: 10.3390/antiox7100134
Accepted: 25 September, 2018; Published: 3 October, 2018

ABSTRACT: Maturity stage affects the bioactive compounds as well as the antioxidant capacity in the fruit. This study was designed to identify and quantify carotenoids contents as well as to evaluate vitamin E, vitamin C, antioxidant capacity and total phenolic compounds of *R. rugosa* hips at different degrees of ripeness. HPLC analysis showed different types of carotenoids at different stages of maturity of rugosa hips with significant differences ($P < 0.05$), where the maximum concentration was observed at late harvesting. In rugosa hips investigated, only α-tocopherol was detected, the maximum concentration of both vitamin E and vitamin C was obtained in the orange hips with significant difference ($P < 0.05$). On the other hand, the highest hydrophilic and lipophilic TEAC values, as well as total phenolic contents, were determined in the mature hips (red colour) with significant difference ($P < 0.0001$) and ($P < 0.001$) respectively, whereas ORAC showed lower activity in the mature hips with significant difference ($P < 0.05$). Late harvesting is recommended if a high content of carotenoids is desired, while harvesting should be carried out earlier if a higher vitamin E and vitamin C content is desired, which in turn affects the antioxidants capacity.

Work share:

Ahlam Al-Yafeai:	Identification and quantification of carotenoids and vitamin E in rugosa hips by HPLC, quantification of vitamin C, measurement of total phenolic compounds and antioxidant capacity, statistical analysis and preparation of the manuscript. Total share: 80%
Peter Bellstedt:	NMR analysis of rubixanthin Total share: 10%
Volker Böhm:	Correction of the manuscript Total share: 10%

4 Discussion

4.1 Identification and quantification of bioactive compounds in rosehips

As mentioned in the literature review, rosehips are a good source for meeting the increasing demand for natural raw materials in food and health-related products. Thus, depending on the product, the differences between species as well as during ripening of the rosehips must be considered. Due to the fact that the majority of the rose hips used for commercial products are harvested from wild populations of plants, specific quality aspects such as content of natural vitamins and antioxidants are difficult to maintain in the raw material. Although rosehips have more recently attracted attention because of their potential health benefits, there is little information about the changes of antioxidants properties in different rosehip products as well as at the different ripening times, especially in *R. rugosa.*

Our research was aimed to investigate authenticity of raw material used in rosehip products and also draw attention to *R. rugosa* because most products are based on the hips of *R. canina,* although they are small compared with the rugosa hips. Furthermore, studies of ripening times are of special interest because they allow the identification of the optimum point of maturity for harvesting which helps to improve levels of bioactive compounds. Although the damage and loss of free carotenoids during the saponification procedure could not be completely avoided, the saponified extracts were used for identification and quantification of carotenoids, because of the absence of esterified xanthophyll standards. The carotenoid pigments in rugosa hips at different ripening times and canina hips as well as canina products have been characterized and identified according to their chemical properties and

chromatographic and spectroscopic characteristics (UV-vis and MS). Due to the polarity, esterified carotenoids appeared latest in the chromatogram, by de-esterification identification was possible as the xanthophylls appeared in the beginning of the chromatogram. Reversed phase HPLC chromatograms corresponding to the saponified extracts of raw materials of rosehips of *R. rugosa* and *R. canina* were almost comparable.

Our findings revealed that carotenoids in *R. rugosa* and *R. canina* rosehip extracts are (*all-E*)- and *(Z)*-β-carotene, (*all-E*)-α- and (*all-E*)-β-cryptoxanthin, (*all-E*)-lutein, (*all-E*)- and (*Z*)-lycopene, (*all-E*)- and (*Z*)-rubixanthin, (*all-E*)-violaxanthin and (*all-E*)-zeaxanthin, being almost comparable with results of (Razungles *et al.*, 1989 & Andersson *et al.*, 2011). The HPLC analysis showed variations in carotenoid contents with significant differences ($P < 0.05$) between these two species of rosehips, in different products as well as at different ripening degrees of *R. rugosa*. The variations in carotenoid contents have been influenced by ecotype and growing conditions, and in products were affected by type of processing. The low carotenoid contents in canina puree may vary under conditions of humidity and temperature during the processing. Whereas higher carotenoid contents in canina powder could be due to the higher dry matter. Andersson *et al.* (2011) found a straight correlation between weather and carotenoid contents in rosehips.

Therefore, for practical implications of the quality of the raw material, it is important to harvest at proper time. To obtain high amounts of most carotenoids in rosehips, harvesting should be carried out after periods of warm and sunny weather. In this study, the carotenoid contents varied during the maturity of rugosa hips, the high concentration was achieved at mature stage (red colour) due to the accumulations of *(all-E)*-β-carotene, *(all-E)*-rubixanthin and *(all-E)*- and *(Z)*-lycopene. The present

findings seem to be consistent with Fraser *et al.* (1994) who showed that the concentration of carotenoids increased in tomato during ripening between 10- and 14-fold mainly due to the accumulation of lycopene. The total carotenoid contents were lowest in the immature stage and showed a pattern of continually increasing accumulation until the final stage of maturity, as was observed for lycopene. This is likely a result of the higher contribution of lycopene to total carotenoids, lycopene is the major carotenoid in mature rosehips (Böhm *et al.*, 2003 & Al-Yafeai *et al.*, 2018). In the same vein, Staffan *et al.* (2008) have reported that the total content of carotenoids increased more than 10-fold during the eight weeks of ripening of *R. spinosissima*, whereas in other rose hip species the contents of carotenoids increased 1.3-2.6 times during the five-week study period. Interestingly, in rugosa hips a significant increase in the total carotenoid contents (1.0-fold) was observed at orange colour, whereas 1.5-fold was observed at mature stage.

Phytoene serves as a precursor of lycopene from which several other carotenoid compounds are synthesized (Li & Yuan, 2013). In accordance with these explanations, the present study has shown a significant decrease in the phytoene contents within the maturity process. Even though, xanthophylls such as violaxanthin, lutein, zeaxanthin and rubixanthin and carotenes such as β-carotene and lycopene were found at all stages of maturation, concentrations tended to change at different ripening stages. Likewise, (*all-E*)-β-carotene was intensively increased in the orange-red stages of maturity. In accordance with the present results, previous studies have demonstrated higher (*all-E*)-β-carotene content in the red stage in all commercial cultivars of tomatoes (Kotikova *et al.*, 2011).

Such differences in the accumulation of (*all-E*)-β-carotene within ripening stages were probably due to the different functions. In immature fruits, *(all-E*)-β-

carotene serves a primary function and is involved in the process of photosynthesis as a photoprotective antioxidant contained in the cores of both photosystems (Cazzaniga *et al.*, 2016). Moreover, (*all-E*)-β-carotene may assume a secondary function and, along with lycopene, contributes to fruit colour (Kotikova *et al.*, 2011).

For more special identification and quantification, (*all-E*)- and (*Z*)-lycopene occurring in rosehips species as well as at different ripening times have been characterized and identified in both unsaponified and saponified extracts by using an isocratic C30-HPLC method, based on comparison with reference compounds (*all-E*)- and (*Z*)-lycopene at 470 nm. Several peaks were subsequently identified as certain geometrical isomers according to the visible spectral data and retention times in HPLC. Corresponding to the maxima absorbance; a hypochromic shifts was observed (at most a 7 nm shift for λ2). The ultraviolet spectrum of (Z)-lycopene moves 5~10 nm towards the shorter wavelengths. Based on comparison with reference compounds the peaks were identified as *(13Z)*-, *(9Z)*-, *(all-E)*- and *(5Z)*-lycopene respectively (Al-Yafeai *et al.*, 2018), where *(5Z, 5'Z)*-lycopene was previously described (Honda *et al.*, 2015). These findings are in agreement with Zhong *et al.* (2016) who referred that rosehips of *R. rugosa* contained three types of (*Z*)-lycopene. Statistical analysis revealed significant differences ($P < 0.05$) in lycopene contents in both rosehips species as well as during rugosa hips ripening times.

Our study provides further evidence for the effect of processing on the relative lycopene contents in each canina product, a decrease in the relative content of *(all-E)*-lycopene was noted to 62% and 66% in canina powder and puree, respectively, compared with the relative content of *(all-E)*-lycopene in raw canina rosehips (69%). The decrease in relative (*all-E*)-lycopene contents is offset by an increase in relative contents of (*Z*)-lycopene isomers in canina products (Al-Yafeai *et al.*, 2018). The

increasing of relative contents of (*Z*)-isomers of lycopene during food processing was previously investigated by Honda *et al.* (2017). On the other hand, (*all-E*)-lycopene contents showed an increasing pattern of accumulation based on total carotenoids during the ripening. A possible explanation for this might be the transition of chloroplasts into chromoplasts. In contrast, (*Z)*-lycopene showed the opposite trend of accumulation compared to *(all-E)*-lycopene, such that its concentration decreased based on total carotenoids in fruits across the ripening stages.

(5′Z)-Rubixanthin (gazaniaxanthin) (Figure 1) has been described as the main *(Z)*-isomer of rubixanthin as well as one of the important features that characterize *R. rugosa* compared to *R. canina* (Al-Yafeai *et al.*, 2018). Gazaniaxanthin was first isolated by Schön, (1938) from flowers of Gazania rigens, where it occurred together with rubixanthin. The HPLC results obtained from the isomerization of *(all-E)*-rubixanthin in $CDCl_3$ by using sun light showed the spectra with maximum absorbance at 463.3 with retention times at 34.35 min. The UV spectrum is characterized by the appearance of a new maximum around 330-350 nm (cis-peak), ultraviolet spectrum moved 5-10 nm towards the shorter wavelengths and this finding is comparable to recent results (Honda *et al.*, 2015 & Huawei *et al.*, 2014).

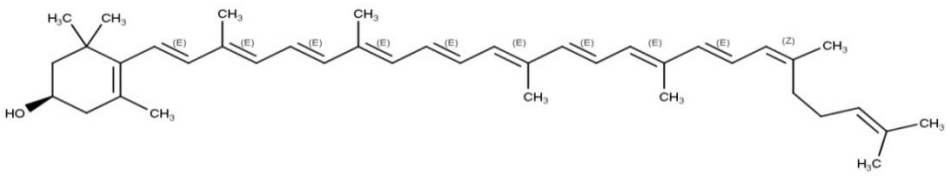

Figure 1. Structure of (5′Z)-rubixanthin.

The NMR data suggest that exposure of (*all-E*)-rubixanthin to sunlight result in an isomerisation of the very last double bond of the conjugated double bond system in the aliphatic chain of rubixanthin, which forms (*5′Z*)-rubixanthin. The ^{1}H-spectra of

(*all-E*)-rubixanthin after exposition to sunlight exhibited two new signals that appeared in the aliphatic region. Since the multiple signals at 5.13 ppm belong to the terminal CH proton of (*all-E*)-rubixanthin, we speculated that the new signal at 5.17 ppm is indicative for the rearrangement of the 5′ double bond from E to Z configuration. Based on selective COSY and TOCSY spectra (Figure 2) that probe for neighbouring protons inside the same spin system, the second new signal of the light-induced isomer could be assigned to the CH_2 group terminal to the 5′ double bond. In (*all-E*)-rubixanthin, both CH_2 groups between the 5′ double bond and the CH group have similar chemical/electronic environment, resulting in a common signal at 2.14 ppm with an integral of 4. Upon light-induced isomerisation, the signal of the CH_2 group adjacent to the 5′ double bond slightly shifted downfield to 2.24 ppm. This shift can be attributed to the emerged spatial proximity of a CH_3 group upon isomerisation of rubixanthin. These findings are consistent with those of Schön (1938) and Arpin & Liannen-Jensen (1968) who suggested that gazaniaxanthin is the corresponding 5′-cis isomer of (*all-E*)-rubixanthin.

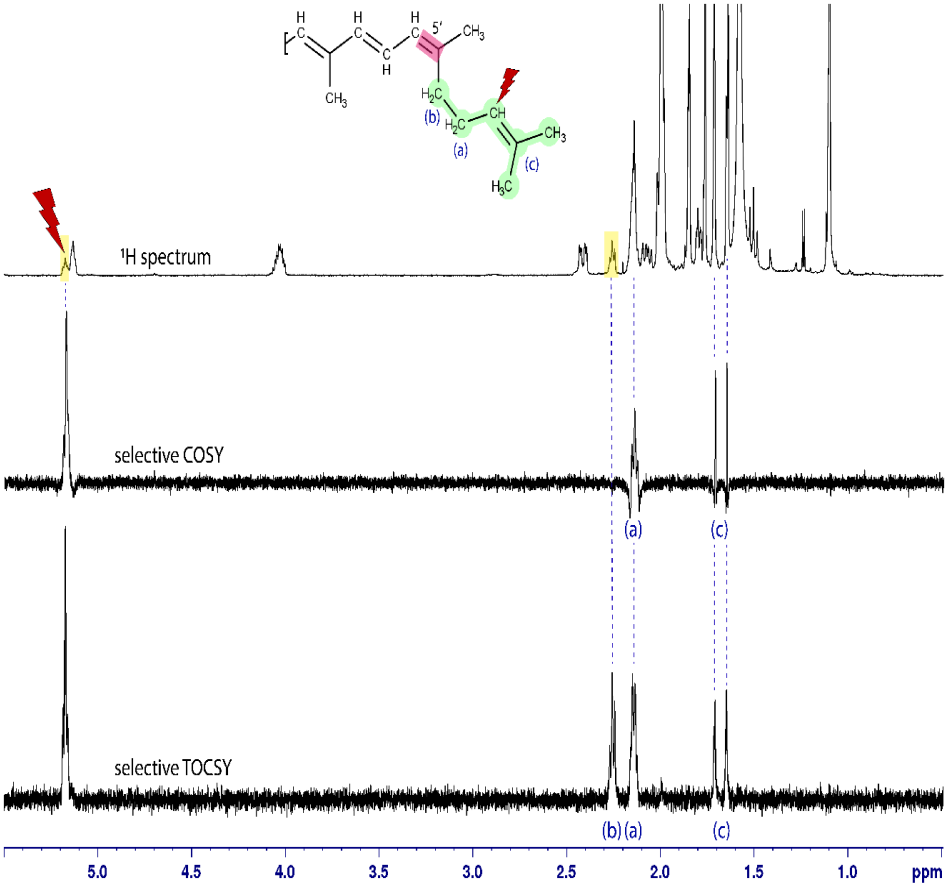

Figure 2: Comparison of 1D COSY and 1D TOCSY spectra selectively exciting the terminal CH group of the light-induced rubixanthin isomer, selective excitation is marked by a red flash. The two new signals in the aliphatic region of the proton spectra, belonging to the light-induced isomer, are highlighted with yellow boxes. The terminal region of the rubixanthin structure is given at the top, the (5′Z) double bond is marked in pink.

4.2 Identification and quantification of vitamin E

The total amounts of tocopherols were not exceptionally high in the present study; α- and ɣ-tocopherol were the main isomers in canina raw material as well as in canina products, being comparable with Andersson *et al.* (2012). In contrast, α-tocopherol was the main isomer of tocopherol in rugosa hips. This finding supports our previous research (Al-Yafeai *et al.*, 2018). Statistical analysis revealed significant differences ($P < 0.001$) in vitamin E contents in both rosehips species as well as in canina products.

On the other hand, α-tocopherol contents are likely to vary during the ripening period; the maximum concentration was achieved in the orange hips with significant difference ($P < 0.05$). The present findings seem to be consistent with Andersson *et al.* (2012) who found that the amounts of total tocopherols and vitamin E activity decreased in rose hips during ripening, but the change was relatively small and limited. Collakova & Dellapenna (2003) have reported that only α- and γ-tocopherol were found in the fleshy parts of the rosehips. The tocopherols are suggested to be synthesized by the action of γ-tocopherol methyl-transferase to either γ- or δ-tocopherol, which can be further synthesised to α- and β-tocopherol, respectively. The tocopherol concentration in rosehips is related to the presence of oil (different fatty acids) (Asplund, 2002), which may contribute to the stability of berries at later stages of development. α-Tocopherol is the most potent tocopherol in quenching singlet oxygen (Zadernowski *et al.*, 2003). Moreover, Falk *et al.* (2002) suggested that oxidative stress may induce increasing of the tocopherol levels in the plant.

4.3 Quantification of vitamin C

As mentioned in the literature review, one of the most important features in rosehips is the high content of vitamin C (Erenturk *et al.*, 2005). Generally, rosehips are considered to be the most abundant natural source of vitamin C, the contents ranged between 200 and 2800 mg/100 g (Uggla *et al.*, 2003). The preliminary analysis of AA in rugosa hips at different ripening times revealed that the contents ranged between 798 mg/100 g and 1090 mg/100 g. It is encouraging to compare these findings with that found by Ercisli, (2007) who showed that contents of AA in the fresh fruits of rose species were between 706 mg/100 g and 974 mg/100 g.

According to the results, the amounts of AA varied greatly at three maturity stages of rugosa hips with a significant difference ($p < 0.05$). The highest contents of vitamin C were achieved at the half-ripe stage (orange colour) with 14% of increasing, whereas these contents decreased in the fully-ripe hips (red colour) by 16%. The present findings seem to be consistent with Zhang *et al.* (2006) who found that the AA contents increased at the beginning and middle of fruit growth and decreased after the development of fruits colour. Rousi & Aulin (1977) reported a decreasing trend in vitamin C contents, accompanied by a steady increase in the fresh weight of the berries. On the other hand, the decrease in vitamin C contents in plants may also be the result of the environmental oxygen level, the amount of light reaching the plants, variations in endogenous plant growth regulators and the temperature (Dogan & Kazankaya, 2006).

Further analysis between rosehips species showed that rugosa hips have high contents of vitamin C (798 ± 37 mg/100 g) compared to canina hips (423 ± 30 mg/100 g) (Table 1), where the vitamin C contents in canina puree as well as in canina powder were 245 ± 14 mg/100 g and 864 ± 37 mg/100 g, respectively with ($P < 0.001$). Several

factors affecting the vitamin C contents in fruits include genotype, preharvest cultural practices, climatic conditions, fruit maturity, harvesting procedures and postharvest management (Porat *et al.*, 2004 & Marcilla *et al.*, 2006) as well as handling and storage, type of container (Naggy, 1980). The climate and environmental conditions affect also the vitamin C contents, increasing the acidity in the citrus fruits leading to increased vitamin C levels. High nitrogen fertilizer rates can lower vitamin C levels in citrus fruits. Proper potassium levels are also needed for a good vitamin C level. Areas with cool nights produce citrus fruits with higher vitamin C levels. In contrast, hot tropical areas produce fruit with lower levels of vitamin C (Padayatty *et al.*, 2003).

AA is synthesized from carbohydrates precursors. Therefore, a lower concentration of sugars in shaded fruit inside the canopy could contribute to lower levels of AA compared to sun-exposed outside fruit (Valpuesta & Botella 2004 & Zhan *et al.*, 2013). On the other hand, Magwaza *et al.* (2013) reported a positive correlation between AA and sucrose and glucose, confirming the role of these sugars, especially glucose, as a primary substrate for AA synthesis pathway. Similarly, as mentioned above, vitamin C contents in different fruits are known to be modulated during fruit development. In tissues of citrus fruit, AA content and concentration is uneven, varying with the stage of fruit development. In fruit such as oranges, tangerines and grapefruits vitamin C contents decreased with maturity, indicating that early harvest or mid-harvest fruit have more AA than late-season fruit (Yang *et al.*, 2011).

4.4 Total phenolic compounds and antioxidants capacity

Among the phytochemical compounds, phenolic compounds and flavonols are regarded as major functional food components. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing

agents, hydrogen donors, singlet oxygen quenchers (Balasundram *et al.*, 2006). Statistical analysis revealed significant differences in the TP contents ($P < 0.0001$) between rosehips species, products and at different maturity stages of rugosa hips. Interestingly, there was a positive correlation between TP and TEAC, where significant differences ($P < 0.001$) have been found in the TEAC activities in rosehips species, products and at the maturity stages of rugosa hips

Table 1. Antioxidant capacity, total phenolic contents, and ascorbic acid in *R. rugosa* and *R. canina* as well as in canina products

Sample	Ascorbic acid [mg/100 g]	H-TEAC [mmol/100 g]	H-ORAC [mmol/100 g]	Total phenolic [mg/100 g]	L-TEAC [mmol/100 g]
R. rugosa	798 ± 37^{c}	15 ± 1^{a}	23 ± 0.3^{a}	1327 ± 43^{a}	4.4 ± 0.3
R. C. R	423 ± 30^{b}	25 ± 2^{b}	26 ± 3.0^{a}	2318 ± 89^{c}	4.0 ± 0.2
R. C. Pr.	864 ± 37^{d}	73 ± 4^{c}	93 ± 10^{b}	5494 ± 41^{d}	0.0 ± 0.0
R. C. Pu	245 ± 14^{a}	20 ± 2^{a}	23 ± 3.0^{a}	1829 ± 18^{b}	0.0 ± 0.0
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P \geq 0.05$

Data are expressed as mean ± SD (n = 3). One-way ANOVA with Student-Newman-Keuls post-hoc test, different letters a/b/c/d within column indicate significant differences between samples ($P < 0.05$).

Canina raw hips and products showed a higher rate of TP as well as TEAC compared with rugosa hips. Likewise, the maturity stage affects, where the highest increases were achieved at the mature stage (red colour) with the rate of 21% for TP and 67% for TEAC. These results seem to be consistent with research done by Ilahy *et al*. (2011). TP accumulation in plants can be affected by genetic factors, environmental and cultural conditions and also various stresses (Parr & Bolwell 2009). These factors can explain the data differences reported within the scientific studies. In the same vein, a decrease in ORAC values has been observed as the ripeness increased. These results

may be explained by the fact that Folin-Ciocalteu and the radical scavenging method ABTS share the same reaction mechanism (electron transfer), whereas the ORAC method is based on hydrogen atom transfer reactions. The present findings seem to be consistent with Rodríguez *et al.* (2016) who found the antioxidants capacity measured by ORAC significantly decreased as the palm fruit became ripe.

On the other hand, an increase in antioxidant capacity of lipophilic extracts was observed with statistical significance ($P < 0.0001$) during the ripening stages of rugosa hips. The increase was progressed during maturity with (38%) in orange colour and with (450%) at full maturity (red colour). This finding confirms our previous results of accumulation of carotenoids during ripening.

4.5 In vitro bioaccessibility of lipophilic micronutrients

Bioavailability of carotenoids is associated with several influential factors, including the carotenoids properties, food matrix and food processing, ingested fat as well as physiological variations connected to digestion (Kotake-Nara & Nagao 2012). Therefore, knowledge about the influence of these factors on *in vitro* accessibility of carotenoids is fundamental, when evaluating the bioavailability potential of carotenoids from rosehip matrices based on *in vitro* results. Although rosehips have more recently attracted attention because of their potential health benefits, no study has investigated the bioaccessibility of carotenoids and vitamin E from rosehip raw materials as well as its products. In this context, the static *in vitro* release of carotenoids from food matrices and their subsequent incorporation into micelles is simulated by such models (Hedré *et al.*, 2002; Reboul *et al.*, 2006 & Werner & Böhm 2011). Mild heating in the initial phase, in addition to extending digestion times during the stomach phase and intestinal

phase, according to the standardized protocol of Minekus *et al.* (2014), were the main modifications in the new model.

4.5.1 Initial phase

Carotenoids are lipophilic micronutrients, located inside the chromoplast organelles in a specific substructure of crystalline, membranous or globular nature embedded in a cellular structure (Schweiggert *et al.*, .2012). Crushing the raw materials helps to release carotenoids by reducing particle size and thus promotes bioaccessibility. Furthermore, amount and type of lipids seem to affect carotenoid bioavailability. In this study, 0.25 ± 0.05 mL of olive oil (main fatty acid: oleic acid (C18:1)) was added, followed by incubation for 1 h at 40 °C with shaking at 250 rpm.

There are similarities between the attitudes expressed by Huo *et al.* (2007) and Salvia-Trujillo *et al.* (2013) who showed an increase in the bioaccessibility of lycopene and β-carotene with increasing fatty acyl chain length (C18:1 > C8:0 > C4:0) upon addition of oil to a salad meal. This was attributed to the greater solubilization capacity of mixed micelles containing long-chain fatty acids. Additionally, thermal processing with 40 °C facilitated the transfer of carotenoids from the cells to the olive oil droplets through weakened plant wall structure. Hedrén *et al.* (2002) reported that thermal treatments promoted the disruption of the food matrix, leading to an increase in β-carotene bioaccessibility in carrot pieces.

4.5.2 Carotenoids contents

Although the contents of carotenoids and vitamin E in rosehip products as well as tomato paste were determined before in a simulated digestion model, this may not necessarily reflect their bioaccessibility. Undoubtedly, the untreated sample was used

to determine the stability of carotenoids during simulated digestion. It was calculated by the contents of substances in the digesta (solid residue + supernatant) in relation to contents in the undigested food. The undigested samples were extracted in the same way as supernatants and residues. Overall, the results of carotenoids and vitamin E were almost comparable to (Al-Yafeai *et al.*, 2018 & Andersson *et al.*, 2011), (*all-E)*- and *(Z)*-lycopene varied between (8 ± 2 mg/100 g to 5 ± 1 mg/100 g) and (8 ± 1 mg/100 g) in rosehip products respectively. In contrast, the concentrations of *(all-E)*-lycopene and *(Z)*-lycopene in tomato paste were 28 ± 3 mg/100 g and 10 ± 12 mg/100 g respectively.

4.5.3 Vitamin E contents

As previously reported, α- and γ-tocopherol were the main tocopherol isomers in *R. canina* rosehip raw materials and products, whereas in *R. rugosa*, only α-tocopherol was determined. The total concentration of vitamin E varied between 32 ± 3 µmol/100 g and 4 ± 0.1 µmol/100 g. On the other hand, vitamin E analysis in tomato paste revealed α- and γ-tocopherol but no tocotrienols and total concentration of vitamin E was 9 ± 1 µmol/100 g.

4.5.4 Pectin contents

Determination of pectin contents may help in explaining the results. In the current analysis, raw hips as well as products already had high contents of pectin. In this context, *R. canina* raw hips contained 2.0 ± 0.01 g GA/100 g compared with *R. rugosa* raw hips with 1.7 ± 0.01 g GA/100 g. The difference in pectin contents depends on the ecotype as well as on ripening time. A high content of pectin in canina products was comparable to Yildiz, & Alpaslan (2012). In contrast, tomato paste had a lower content

of pectin 0.4 ± 0.0 g GA/100 g. Pectin contents in samples were reduced significantly ($P < 0.05$) after being treated with the enzyme mixture Fructozym P6-XL.

Moreover, the results also showed incomplete degradation of pectin in samples after treatment with Fructozym P6-XL, perhaps because fruit cell walls are often pectin-enriched. Grassin & Fauquembergue (1996) reported that residual pectins and hemicelluloses in strawberries and raspberries bind to phenolic substances and proteins during processing and storage, resulting in irreversible complexes that enzymes cannot cleave. On the other hand, different relative degradation rates in the treated samples were observed. A possible explanation for this might be the type of carbon source and structure of pectin substrates, influencing the enzyme activity profile. PL prefers to hydrolyze high-esterified pectin. In contrast, PG is a pectin-depolymerizing enzyme, cleaving α-(1−4)-glycosidic linkages in polygalacturonic acid by trans-elimination, exo-PG and endo-PG more often cleave low-esterified pectin. Furthermore, PME catalyzes de-esterification of methoxyl groups of pectin forming pectic acid. The enzyme acts preferentially on a methyl ester group of a galacturonate unit next to a nonesterified galacturonate unit.

4.5.5 Pectin and Bioaccessibility

Although soluble fiber consumption has widely recognized health benefits, the impact of pectin and other dietary fibers on the bioavailability and metabolism of other nutrients, including lipids, is still under investigation. Some *in vitro* studies showed for pectin a potential to impact lipid digestion processes (Zhang *et al.*, 2015) which can affect the carotenoid's bioaccessibility. Following the addition of the enzyme mixture Fructozym P6-XL, the bioaccessibility of carotenoids from rosehips significantly increased. The average relative increase was lower for ((*all-E*))-β-carotene (+21%)

compared with ((*all-E*))-lycopene (+50%) and (*all-E*)-rubixanthin (+48%). In contrast, in tomato paste, a strong evidence of bioaccessibility of individual carotenoids was found after treatment with Fructozym P6-XL ($P < 0.001$). Likewise, adding pectin to tomato paste caused significant decrease of the bioaccessibility of *(all-E)*-lycopene (-68%), *(Z)*-lycopene (-60%) and *(all-E)*-β-carotene (-49%), these results match those observed in earlier studies (Cervantes-Paz *et al.*, 2016).

Pectin can affect bioaccessibility through two mechanisms, the passive absorption in the small intestine may be blocked as a result of pectin action on the lipids and bile salt molecules, thereby avoiding micelle formation with carotenoids. Furthermore, pectin increases the viscosity of the intestinal content. Thus, absorption of antioxidants was reduced perhaps due to lower activity of enzymes and increased difficulty in contacting intestinal enterocyte (Xu *et al.*, 2015). The effects of pectin on bioaccessibility and bioavailability depend on different factors; such as pectin degree of methylation (DM), distribution of nonmethylesterified galacturonic acid residues, molar mass, linear charge density and hydrophobicity. Kyomugasho *et al.* (2015) showed that in regions containing pectin with a higher DM, cell adhesion is probably weaker, and thus, tissues are more easily disintegrated into smaller particles. On the other hand, pectin with a lower DM is more strongly bound in cell walls, and thus, regions rich in low DM pectin are not easily disrupted resulting in larger particles. In general, high-DM pectin increases the lipolysis, bile salt binding, and micellarization of polar carotenoids (Cervantes-Paz *et al.*, 2016). However, low-DM pectin can reduce the levels of calcium involved in the lipolysis and generation of lipid digestion products for micelle formation (Kyomugasho *et al.*, 2015).

4.5.6 Effects of food processing on bioaccessibility of carotenoids

Food matrix structure is one of the most important factors that affect the bioaccessibility of carotenoids. The most interesting finding was that there were differences in the efficiency of carotenoids micellarization in the two rosehip species investigated. The bioaccessibility of total lycopene in rugosa hips was higher than that in canina hips. These findings are supported by Stinco *et al.* (2012). As mentioned above, pectin food matrix-related factors may hinder carotenoids bioaccessibility. *R. canina* hips had higher pectin contents compared with *R. rugosa* hips, this could be a reason for the relatively low carotenoids bioaccessibilities of the canina hips.

In addition, samples also include three different products of *R. canina* (powder, jam and puree). Results for (*all-E*) and (*Z*)-lycopene were highly variable in different products, whereas the accessibilities of (*all-E*)-β-carotene and (*all-E*)-rubixanthin were almost comparable. The order of lycopene bioaccessibilities was determined to be R.C.Pr > R.C.Ja ≥ R.C.Pu. A possible explanation for this might be related to food processing. Food processing with breaking of the natural matrix may lead to higher bioavailability (Parada & Aguilera 2007). Consequently, the effect of particle size reduction is expected to arise from disintegration of the food matrix and reduction in the potential plant cell wall barrier toward digestion (Bohn *et al.*, 2015). Accordingly, the solubility is enhanced by increasing the surface area for dissolution, thus improving activity of the enzymes and permeation of bile salts.

The positive effect of food processing on carotenoid bioaccessibility is in accordance with *in vivo* studies on carotenoid bioavailability (Van Het Hof *et al.*, 2000) and confirms that eating processed vegetables improves carotenoid bioavailability. Interestingly, further comparison between the bioaccessibilities of individual

carotenoids in rosehip and tomato paste showed that tomato paste had a higher carotenoids bioaccessibility. The present findings seem to be consistent with the results of an *in vivo* study (Unpublished data) (Brose, 2010).

Furthermore, Schweiggert & Carle (2017) showed tubular or globular-tubular chromoplasts for hips of *R. rugosa*, partially containing lipid-dissolved but mostly liquid-crystalline carotenoids. Consequently, the globular-tubular deposition form might be more favorable than the protein-complexed and solid-crystalline forms, respectively. In contrast, crystalloid chromoplasts were previously described in red tomato (Schweiggert *et al.*, 2011). The low bioaccessibility was affected by the deposition of carotenoids in protein pigment complexes and crystalline aggregates, respectively (Schweiggert *et al.*, 2014). On the other hand, Zhou *et al.* (1996) have previously reported that the crystalline state of carotenoids was associated with their poor bioavailability. These two reasons as well as high pectin contents in rosehips may explain the relatively low bioaccessibility in rosehips compared to tomato paste. According to Cooperstone *et al.* (2015) lycopene was markedly more bioavailable from tangerine than from red tomato juice, consistent with a predominance of (*Z*)-lycopene isomers and presence in chromoplasts in a lipid dissolved globular state. These reasons in addition to the profile structure of cis-isomers could be a possible explanation for the more efficient micellarization of cis-isomers compared to trans-isomers.

4.5.7 Effect of carotenoid properties

Regardless of the sample type or the treatment, the carotenoids showed different bioaccessibility. Overall, the bioaccessibility decreased in the following order: *(all-E)*-rubixanthin ≥ (*all-E)*-β-carotene > *(Z)*-lycopene > (*all-E*)-lycopene. Carotenoid hydrophobicity and transfer efficiency were inversely related. Xanthophylls are more

efficiently transferred into micelles and thus show a higher bioaccessibility than the hydrocarbons β-carotene or lycopene (Schweiggert *et al.*, 2012). In agreement with this hypothesis, the micellarization of rubixanthin increased after treatment with Fructozym P6-XL in all rosehip samples. However, an unexpected result was observed in the present study, the bioaccessibility of (*all-E*)-β-carotene was greater than that of (*all-E*)-rubixanthin in all untreated rosehip samples. A possible explanation for these results: the hydrophobicity is not the only factor affecting the carotenoids bioaccessibility. In the same vein, Sólyom *et al.* (2014) showed the following order of bioaccessibilities: β-carotene > γ-carotene > lycopene ≈ β-cryptoxanthin > rubixanthin after delivery of carotenoids to an *in vitro* digestion model.

Furthermore, (*all-E*)-lycopene had a significantly lower bioaccessibility compared to (*all-E*)-β-carotene in all samples with different treatments. These findings seem to be consistent with other research (Zhou *et al.*, 1996). Lycopene is more lipophilic than β-carotene because of its acyclic structure without a β-ionone ring. On the other hand, the interactions between carotenoids have been demonstrated. During micellar formation, the competition between lycopene and β-carotene has been recorded (Fernández-García *et al.*, 2007). The favored β-carotene transfer into the micelles could also hinder lycopene incorporation. Further analysis showed that the efficiency of micellarization of (*Z*)-lycopene was significantly greater than that of (*all-E*)-lycopene. These results match those observed in earlier studies by Ferruzzi *et al.*, (2006) who found that the profile structures of cis-isomers were micellarized more efficiently than trans-isomers. The (*Z*)-lycopene isomers are less likely to crystallize, more oil/hydrocarbon soluble, compared to (*all-E*)-lycopene. During a simulated *in vitro* digestion model, the recovery rates of carotenoids were determined, the rates of

stabilities were >70% with average 80%. Oil droplets were removed from the aqueous fraction by filtration, resulting thus in a loss in carotenoids in supernatant and residue.

4.5.8 Vitamin E Bioaccessibility

Vitamin E bioaccessibility significantly increased after treatment with Fructozym; there are similarities between these findings and earlier observations with carotenoids. The stability of vitamin E during simulated digestion was >78% and averaged 82%. In agreement with Reboul *et al.* (2006), the bioaccessibility of tocopherols from different foodstuffs was highly variable. As mentioned above, the bioaccessibility of nutrients depend on the physical properties of the food matrix.

Dietary tocopherols are mostly present in the free forms (except in fortified food). Dietary carotenoids may be present as protein-bound, ester and free forms. This may explain the higher bioaccessibility of vitamin E compared with lycopene. These results match those observed in earlier studies (Reboul *et al.*, 2006). Although the study has successfully gone some way toward enhancing our understanding of correlations between the pectin contents, food processing and bioaccessibility, it has raised some new questions in need of further investigation. Our results confirm that pectin has a major effect on the bioaccessibility, significantly increasing the bioaccessibilities of carotenoids and vitamin E after pectin hydrolysis by using Fructozym P6-XL.

5 Summary

5.1 Background

The hips of Rosa species have received more attention in recent years due to their high contents of antioxidants. The genus Rosa contains over 100 species, which are widespread and different in their appearance as well as in the chemical composition. Indeed, the maturity stage affects bioactive compounds and antioxidant capacity in rosehips. On the other hand, the limited bioavailability of antioxidants present in food from fruit and vegetable matrices is determined by their low bioaccessibility due to the physical and chemical interactions of the antioxidants with the indigestible polysaccharides of cell walls.

5.2 Objectives

Identification and quantification of carotenoids, vitamin E and vitamin C as well as investigation of biological activities in both rugosa and canina hips. A second goal was to study the factors that influence the bioaccessibilities of carotenoids and vitamin E in rosehips and tomato paste.

5.3 Methods

Carotenoids and vitamin E were extracted in both rosehips species, the identifications were performed by comparison with external standards or were tentatively identified by comparison of retention times and DAD absorbance spectra as well as mass spectra. The quantification was carried out by using 5-point calibration curves of external standards. Antioxidant capacity and vitamin C were determined by using spectrophotometric techniques using the methods having been described in the related manuscript. For the second objective, the static *in vitro* digestion model was designed

to study the effect of pectin on bioaccessibility. The experiment was conducted in three groups depending on the pectin contents: (i) control sample, (ii) sample treated with Fructozym P6-XL, (iii) sample with added pectin. The simulation of the digestive process involves a three-step procedure simulating mouth/stomach/small intestine, with conditions generally based on physiological conditions *in vivo.*

5.4 Results

Study 1:

The HPLC analysis showed that carotenoid contents varied between rosehips species as well as at different stages of maturity of rugosa hips, statistical analysis revealed that rugosa hips had higher contents of carotenoids compared with raw canina hips with significant differences ($P < 0.05$). On the other hand, maturity stage affects the bioactive compounds in rugosa hips, the maximum concentration was observed at late harvesting. The high concentration of lycopene appeared to be a characteristic of the Rosa species, the ripening process of hips is associated with increase in *(all-E)*-lycopene content with significant difference ($P < 0.001$). In contrast, the maximum concentration of *(Z)*-lycopene was achieved in the green hips with 40% of the whole contents of carotenoids. HLPC analysis of vitamin E showed that α- and ɣ-tocopherol were the main isomers in canina hips, while rugosa hips had only α-tocopherol. Statistical analysis showed a significant difference in vitamin E content ($P < 0.001$) between different hip products with the maximum concentration of each vitamin E isomer in the orange hips with significant difference ($P < 0.05$). In the same way, rosehips are considered to be the most abundant natural source of vitamin C, rugosa hips had a higher concentration of vitamin C compared with canina raw hips with significant difference ($P < 0.0001$). Likewise, depending on the maturity stage of rugosa hips, the maximum concentration

was achieved in the orange hips with significant difference ($P < 0.05$). The highest hydrophilic and lipophilic TEAC values were determined in the mature hips (red colour) with significant difference ($P < 0.0001$) as well as total phenolics contents with ($P < 0.001$), whereas ORAC showed lower activity in the mature hips with significant difference ($P < 0.05$).

Study 2:

The second major finding was that studying the bioaccessibility, following the addition of the enzyme mixture Fructozym P6-XL, the bioaccessibility of carotenoids from rosehips as well as from tomato paste significantly increased ($P < 0.05$). The average relative increase in bioaccessibility from rosehips was lower for (*all-E*)-β-carotene compared with (*all-E*)-lycopene and (*all-E*)-rubixanthin. In contrast, increases of bioaccessibility of α-tocopherol were comparable for rosehip samples and tomato paste.

5.5 Conclusion

This research extends our knowledge of rosehip, confirms previous findings and contributes additional evidence that suggests rosehip as a good source of carotenoids, vitamin E and vitamin C. The variations in contents of these bioactive compounds have been influenced by ecotype, growing conditions and the degree of the maturity, and in the products were affected by the type of processing. Higher contents of carotenoids and vitamin C were observed in rugosa hips compared with canina raw hips. On the other hand, this study has gone some way towards enhancing our understanding of the factors that affect the bioaccessibilities of carotenoids and vitamin E.

6 References

Aboul-Soud, M. A., Al-Othman, A. M., El-Desoky, G. E., Al- Othman, Z. A., Yusuf, K., Ahmad, J. & Al Khedhairy, A. A. (2011): Hepatoprotective effects of vitamin E/selenium against malathioninduced injuries on the antioxidant status and apoptosis related gene expression in rats. Journal of Toxicological Sciences. 36, 285-296.

Abu-Abed, S., Dollé, P., Metzger, D., Beckett, B., Chambon, P. & Petkovich, M. (2001). The retinoic acid-metabolizing enzyme, CYP26A1, is essential for normal hindbrain patterning, vertebral identity and development of posterior structures. Genes & Development, 15(2), 226-240.

Agarwal, M., Parameswari, R. P., Vasanthi, H. R. & Das, D. K. (2012). Dynamic action of carotenoids in cardioprotection and maintenance of cardic health. Molecules, 17, 4755-4769.

Albanes, D., Heinonen, O.P., Huttunen, J. K., Taylor, P. R., Virtamo, J., Edwards, B. K., Haapakoski, J., Rautalahti, M., Hartman, A. M. & Palmgren, J. (1995). Effects of α-tocopherol and β-carotene supplements on cancer incidence in the Alpha-Tocopherol Beta-Carotene cancer prevention study. The American Journal of Clinical Nutrition 62, 1427S-1430.

Albersheim, P., Darvill, A. G., O'Neill, M. A., Schols, H. A. & Voragen, A. G. J. (1996). A hypothesis: the same six polysaccharides are components of the primary cell walls of all higher plants. Progress in Biotechnology, 14:47-55.

Al-Yafeai, A. & Böhm, V. (2018). *In vitro* bioaccessibility of carotenoids and vitamin E in rosehip products and tomato paste as affected by pectin contents and food processing. Journal of Agricultural and Food Chemistry, 66, 3801-3809

Al-Yafeai, A., Malarski, A. & Böhm, V. (2018). Characterization of carotenoids and vitamin E in *R. rugosa* and *R. canina*: Comparative analysis. Food Chemistry, 242, 435-442

Andersson, S. C., Olsson, M. E., Gustavsson, K.-E., Johansson, E. & Rumpunen, K. (2012). Tocopherols in rose hips (Rosa spp.) during ripening. Journal of the Science of Food and Agriculture, 92, 2116-2121.

Andersson, S. C., Rumpunen, K., Johansson, E. & Olsson, M. E. (2011). Carotenoid content and composition in rose hips (Rosa spp.) during ripening, determination of suitable maturity marker and implications for health promoting food products. Food Chemistry, 128, 689-696.

Arpin, N. & Liaaen-Jensen, S. (1969). Carotenoids of higher plants-11 rubixanthin and gazaniaxanthin

Asplund, K. (2002). Antioxidant vitamins in the prevention of cardiovascular disease: A systematic review. Journal of Internal Medicine, 251, 372-392.

Bagherian, H., Ashtiani, F. Z., Fouladitajar, A. & Mohtashamy, M. (2011). Comparisons between conventional, microwave- and ultrasound-assisted methods for extraction of pectin from grapefruit. Chemical Engineering and Processing, 50:1237-43.

Balasundram, N., Sundram, K. & Sammar, S. (2006). Phenolic compounds in plants and agro-industrial by-products: Antioxidant Activity. Occurrence and Potential Uses. Food Chemistry, *99(1),* 191-203

Baltschun, D., Beutner, S., Briviba, K., Martin, H.-D., Paust J., Peters, M., Röver, S., Sies, H., Stahl, W., Steigel, A. & Stenhorst, F. (1997). Singlet oxygen quenching abilities of carotenoids. European Journal of Organic Chemistry, (9), 1887– 1893.

Barros, L., Carvalho, A. M. & Ferreira, I. R. (2011). Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal. Food Research International, 44, 2233-2236.

Bender, D. A. & Mayes, P. A. (2003). Vitamins and minerals. Harper's Illustrated Biochemistry 26 ed. 45: Murray RK, Granner DK, Mayes PA & Rodwell VW, Eds.; Lange Medical Books/McGraw-Hill, New York.

Böhm, V., Fröhlich, K. & Bitsch, R. (2003). Rosehip - a ‘‘new’’ source of lycopene? Molecular Aspects of Medicine, 24, 385-389.

Bohn, T. (2008). Bioavailability of non-provitamin A carotenoids. Current Nutrition & Food Science, 4, 240-258.

Bohn, T., McDougall, G. J., Alegría, A., Alminger, M., Arrigoni, E., Aura, A. M., Brito, C., Cilla, A., El, S. N., Karakaya, S., Martínez-Cuesta, M. C. & Santos, C. N. (2015). Mind the gap - deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites a position paper focusing on carotenoids and polyphenols. Molecular Nutrition & Food Research, 59 (7), 1307-1323.

Borel, P., Tyssandier, V., Mekki, N., Grolier, P., Rochette, Y., Alexandre-Gouabau, M. C., Lairon, D. & Azaïs-Braesco, V. (1998). Chylomicron β-carotene and retinyl palmitate responses are dramatically diminished when men ingest beta-carotene with medium-chain rather than long-chain triglycerides. Journal of Nutrition. 128, 1361-1367.

Brigelius-Flohe, R. & Traber, M.G.(1999). Vitamin E: Function and metabolism. FASEB Journal, 13, 1145-1155.

Britton, G., Liaaen-Jensen, S. & Pfander, H. (1995). Carotenoids. Vol. 1B: Spectroscopy. Basel, Switzerland: Birkhäuser Verlag.

Britton, G., Liaaen-Jensen, S. & Pfander, H. (2004). Carotenoids Handbook. Basel, Switzerland: Birkhäuser Verlag.

Brose, A. Humaninterventionsstudien zur Untersuchung der Intestinalen Absorption von Lycopin und Rubixanthin aus Hagebuttenmark (2010). Diploma Thesis, Friedrich Schiller University Jena, Jena, Germany,

Cazzaniga, S., Bressan, M., Carbonera, D., Agostini, A. & Dall'Osto, L. (2016). Differential roles of carotenes and xanthophylls in photosystem I photoprotection, Biochemistry, 26, 3636-3649.

Cervantes-Paz, B., Ornelas-Paz, J., Pérez-Martínez, J. D., Reyes- Hernández, J., Zamudio-Flores, P. B., Rios-Velasco, V., Ibarra- Junquera, S., Ruiz-Cruz, S. & Ruiz-Cruz. (2016). Effect of pectin concentration and properties on digestive events involved on micellarization of free and esterified carotenoids. Food Hydrocolloids, 60, 580-588.

Christensen, R., Sorensen, L. B., Bartels, E. M., Astrup, A. & Bliddal, H. (2007). Rose-hip in osteoarthritis (OA): a meta-analysis. Annals of the Rheumatic Diseases 66 (Suppl 2), 495.

Christiaens, S., VanBuggenhout, S.,Vandevenne, E., Jolie, R., VanLoey, A. & Hendrickx, M. (2011). Towards a better understanding of the pectin structure–function relationship in broccoli during processing: Part II-analyses with anti-pectin antibodies. Food Research International, 44, 2896-906.

Chrubasik, C., Roufogalis, B. D., Müller-Ladner, U. & Chrubasik, S. (2008). A systematic review on the *Rosa canina* effect and efficacy profiles. Phytotherapy Research, 22:725-733

Collakova, E. & DellaPenna, D. (2003). The role of homogentisate phytyltransferase and other tocopherol pathway enzymes in the regulation of tocopherol synthesis during abiotic stress. Plant Physiology, 133, 930-940.

Cooperstone, J. L., Ralston, R. A., Riedl, K. M., Haufe, T. C., Schweiggert, R. M., King, S. A.,Timmers, C. D., Francis, D. M., Lesinski, G. B.,Clinton, S. K. & Schwartz, S. J. (2015). Enhanced bioavailability of lycopene when consumed as cis-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. Mol. Nutrition Food Research, 59, 658-669.

Cramer, W., Kuper, H., Harlow, B. L. & Titus-Ernstoff, L. (2001). Carotenoids, antioxidants and ovarian cancer risk in pre- and postmenopausal women. International Journal of Cancer, 94(1), 128-134.

Daels-Rakotoarison, D. A., Gressier, B., Trotin, F., Brunet, C., Luyckx, M., Dine, T., Bailleul, F., Cazin, M. & Cazin, J. C. (2002). Effects of *Rosa canina* fruit extracts on neutrophil respiratory burst. Phytotherapy Research 16, 157-161.

D'Ambrosio, D. N. & Clugston, R. D. (2011). Vitamin A Metabolism: An Update. Nutrients, 3(1), 63-103.

Davey, M. W, Auwerkerken, A. & Keulemans, J. (2007). Relationship of apple vitamin C and antioxidant contents to harvest date and postharvest pathogen infection. Journal of the Science of Food and Agriculture, 87, 802-813.

Davey, M. W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J., Strain, J. J., Favell, D. & Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture, 80, 825-860.

Deliorman, O. D., Harteviollu, A., Küpeli, E. & Yesilada, E. (2007). In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. Journal of Ethnopharmacology 112, 394-400.

DellaPenna, D. A (2005). decade of progress in understanding vitamin E synthesis in plants. Journal of Plant Physiology. 162, 729-737.

Dogan, A. & Kazankaya, A. (2006). Fruit properties of rose Hip species grown in lake van basin (eastern Anatolia region). Asian Journal of Plant Science, 5,120-122.

Ercisli, S. (2007). Chemical composition of fruits in some rose (Rosa spp.) species. Food Chemistry, 104, 1379-1384.

Fernández-García, E., Mínguez-Mosquera, M. I. & Pérez-Gálvez, A. (2007). Changes in composition of the lipid matrix produce a differential incorporation of carotenoids in micelles. Interaction effect of cholesterol and oil. Innovative Food Science & Emerging Technology, (3), 379-384.

Erenturk, S., Gulaboglu, M. S. & Gultekin, S. (2005). The effects of cutting and drying medium on the vitamin C content of rosehip during drying. Journal of Food Engineering, 68, 513-518.

Evans, H. M. & Bishop, K. S. (1923). Existence of a hitherto unknown dietary factor essential for reproduction. Journal of the American Medical Association . 81: 889-892. 12.

Everett, S. A., Dennis, M. F., Patel, K. B., Maddix, S., Kundu, S. C. & Willson, R. L. (1996). Scavenging of nitrogen dioxide, thiyl, and sulfonyl free radicals by the nutritional antioxidant β-carotene. Journal of Biological Chemistry, 271(8), 3988-3994.

Falk, J., Krauss, N., Dahnhardt, D. & Krupinska, K. (2002). The senescence associated gene of barley encoding 4-hydroxyphenylpyruvate dioxygenase is expressed during oxidative stress. Journal of Plant Physiology, 159, 1245-1253.

Ferruzzi, M. G., Lumpkin, J. L., Schwartz, S. J. & Failla, M. (2006). Digestive stability, micellarization, and uptake of α-carotene isomers by Caco-2 human intestinal cells. Journal of Agricultural and Food Chemistry, 54, 2780-2785.

Franck, C., Baetens, M., Lammertyn, J., Verboven, P., Davey, M. W. & Nicolai, B. M. (2003). Ascorbic acid concentration in cv. conference pears during fruit development and postharvest storage. Journal of Agricultural and Food Chemistry, 51, 4757-4763.

Fraser, P. D., Truesdale, M. R., Bird, C. R, Schuch, W. & Bramley P. M. (1994). Carotenoid biosynthesis during tomato fruit development. Plant Physiology, 105, 405-413.

Furr, H. C. & Clark, R. M. (1997). Intestinal absorption and tissue distribution of carotenoids. Journal of Nutritional Biochemistry, 8, 36-377.

Gandini, S., Merzenich, H., Robertson, C. & Boyle, P. (2000). Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. European Journal of Cancer, 36, 636-646.

Gokmen, V., Kahraman, N., Demir, N. & Acar, J. (2000). Enzymatically validated liquidchromatographic method for the determination of ascorbic anddehydroascorbic acids in fruit and vegetables. Journal of Chromatography, A 881, 309-316.

Gonzales, I., Escobar, M. & Olivera, P. (1997). Plasma lipids of golden Syrian hamsters fed dietary rose hip, sunflower, olive and coconut oils. Journal of Physiology and Biochemistry, 53, 199-204.

Grassin, C. & Fauquembergue, P. (1996). Fruit juices. In: Industrial Enzymology, 2nd ed.; Godfrey, T., Reichelt, J., West, S., Eds.; Nature Publishing Group: London, pp 225-264.

Groff, J. L., Gropper, S. S. & Hunt, S.M. (1995). The water soluble vitamins. In: Advanced nutrition and human metabolism. West publishing company, Minneapolis, pp. 222-231.

Grusak, M. A. & DellaPenna, D. (1999). Improving the nutrient composition of plants to enhance human nutrition and health. Annual Review of Plant Biology, 50, 133-161.

Güne, M. (2010). Pomological and phenological characteristics of promising rose hip (Rosa) genotypes. African Journal of Biotechnology, 9, 6301-6306.

Guo, D., Xu, L., Cao, X., Guo, Y., Chan, C. O., Mok, D. K. W., Yu. Z. & Chen, S. (2011). Anti-inflammatory activities and mechanisms of action of the petroleum ether fraction of *Rosa multiflora* Thunb. hips. Journal of Ethnopharmacology, 138, 717-722.

Guo, X., Zhao, W., Pang, X., Liao, X., Hu, X. & Wu, J. (2014). Emulsion stabilizing properties of pectins extracted by high hydrostatic pressure, high-speed shearing homogenization and traditional thermal methods: a comparative study. Food Hydrocolloids, 35, 217-25.

Halliwell, B. & Gutteridge, J.M. (2015). Free Radicals in Biology and Medicine; Oxford University Press: New York, NY, USA.

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Remberg, S. F., Wold, A. B., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, J. Ø., Jacobs, D. R.J. & Blomhoff, R. (2002). A systematic screening of total antioxidants in dietary plants1. Journal of Nutrition, 132, 461-471.

Hedrén, E., Diaz, V. & Svanberg, U. (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. European Journal of Clinical Nutrition, 56 (5), 425-430.

Henker, H. (2000). *Rosa* L. In: Conert H. J., Jäger E. J., Kadereit J. W., Schultze-Motel W., Wagenitz G., Weber H. E. (eds), Hegi Illustrierte Flora von Mitteleuropa. Bd. 4/2 C: 1–108. Parey Buchverlag, Berlin.

Holst, B. & Williamson, G. (2008). Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. Current Opinion in Biotechnology, 19, 73-82.

Honda, M., Murakami, K., Watanabe, Y., Higashiura, T., Fukaya, T., Wahyudiono, Kanda, H. & Goto, M. (2017). The E/Z isomer ratio of lycopene in foods and effect of heating with edible oils and fats on isomerisation of (*all-E*)-lycopene. European Journal of Lipid Science and Technology, 119, 1600389.

Honda, M., Takahashi, N., Kuwa, T., Takehara, M., Inoue, Y. & Kumagai, T. (2015). Spectral characterisation of Z-isomers of lycopene formed during heat treatment and solvent effects on the E/Z isomerisation. Food Chemistry, 171, 323-329.

Horvath, G.,Wessjohann, L., Bigirimana, J., Jansen, M., Guisez, Y., Caubergs, R. & Horemans, N. (2006). Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. Phytochemistry, 67, 1185–1195.

Hu, Z. C., Zheng, Y. G., Wang, Z. & Shen, Y. C. (2006). pH Control strategy in astaxanthin fermentation bioprocess by Xanthophyllomyces dendrorhous. Enzyme Microbial Technology, 39(4), 586-590.

Huawei, Z., Xiaowen, W., Elshareif, O., Hong, L., Qingrui, S. & Lianfu, Z. (2014). Isomerisation and degradation of lycopene during heat processing in simulated food system. International Food Research Journal, 21(1), 45-50.

Huo, T., Ferruzzi, M. G., Schwartz, S. J. & Failla, M. L. (2007). Impact of fatty acyl composition and quantity of triglycerides on bioaccessibility of dietary carotenoids. Journal of Agricultural and Food Chemistry, 55 (22), 8950-8957.

Ilahy, R., Hdider, C., Lenucci, M. S., Tlili, I. & Dalessandro, G. (2001). Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. Journal of Food Composition and Analysis, 24, 588-595.

Johnson, L. R. & Gerwin, T. A. (2001). Gastrointestinal Physiology. 6th ed. St. Louis, Missouri: Mosby, Inc., 119-141.

Joshipura, K. J., Hu, F. B., Manson, J. E., Stampfer, M. J., Rimm, E. B., Speizer, F. E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D. & Willett, W. C. (2001). The effect of fruit and vegetable intake on risk for coronary heart disease. Annals of International Medicine, 134, 1106-1114.

Kamal-Eldin, A. & Appelqvist, L. A. (1993). The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, 31, 671-701.

Karakaya, S. & Kavas, A. (1999). Antimutagenic activities of some foods. Journal of the Science of Food and Agricultural, 79, 237-242.

Kotake-Nara, E. & Nagao, A. (2012). Effects of mixed micellar lipids on carotenoid uptake by human intestinal Caco-2 cells. Bioscience, Biotechnology and Biochemistry, 76:875-882.

Kotıkova, Z., Lachman, J., Hejtmankova, A. & Hejtmankov, K. (2011). Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. LWT-Food Science and Technology, vol. 44, 8, 1703-1710.

Krieger-Liszkay, A., Fufezan, C. & Trebst, A. (2008). Singlet oxygen production in photosystem II and related protection mechanism. Photosynthesis Research, 98, 551-564.

Krinsky, N. I. & Johnson, E. J. (2005). Carotenoid action and their relation to health and disease. Molecular Aspects of Medicine 26, 459-516.

Kumarasamy, Y., Cox, P. J., Jaspars, M., Nahar, L. & Sarker, S. D. (2002). Screening seeds of Scottish plants for antibacterial activity. Journal of Ethnopharmacology 83, 73-77.

Kyomugasho, C., Willemsen, L. D. D., Christiaens, S., Van Loey, A. M. & Hendrickx, M. E. (2015) Pectin-interactions and in vitro bioaccessibility of calcium and iron in particulated tomato-based suspensions. Food Hydrocolloids, 49, 164-175.

Lampe, J. W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. American Journal of Clinical Nutrition 70, 475-490.

Lass, A. & Sohal, R. S. (1998). Electron transport-linked ubiquinone-dependent recycling of alpha-tocopherol inhibits autooxidation of mitochondrial membranes. Archives of Biochemistry and Biophysics, 352, 229-236.

Li, L. & Yuna, H. (2013). Chromoplast biogenesis and carotenoid accumulation. Archives of Biochemistry and Biophysics 539, 102-109

Liebler, D. C. (1993). The role of metabolism in the antioxidant function of vitamin E. Critical Reviews in Toxicology, 23, 147-169.

Liu, S., Lee, I. M., Ajani, U., Cole, S.R., Buring, J. E. & Manson, J. E. (2001). Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: the physician's health study. International Journal of Epidemiology, 30, 130-135.

Liu, S., Manson, J. E., Lee, I. M., Cole, S. R., Hennekens, C. H., Willett, W. C. & Buring, J. E. (2000). Fruit and vegetable intake and risk of cardiovascular disease: the women's health study. American Journal of Clinical Nutrition, 72, 922-928.

Lopaczynski, W. & Zeisel, S. H. (2001). Antioxidants, programmed cell death, and cancer. Nutrition Research, 21, 295-307.

Lopez, M., Arce, M., Garrido, J., Rıos, A. & Valcarcel, M. (2004). Selective extraction of astaxanthin fromcrustaceans by use of supercritical carbon dioxide. Talanta, 64 (3), 726-731.

Magwaza, L. S., Opara, U. L., Cronje, P. J. R., Landahl, S., Terry, L. A. (2013). Canopyposition effect on rind biochemical profile of 'Nules Clementine' mandarin fruitduring postharvest storage. Postharvest Biology and Technology, 86, 300-308.

Marcilla, A., Zarzo, M. & Del Río, M. A. (2006). Effect of storage temperature on the flavour of citrus fruit. Spanish Journal of Agricultural Research, 4, 336-344

McClements, D. J. & Decker, E. A. (2009). Controlling lipid bioavailability using emulsion based delivery systems. In McClements, D. J., Decker, E. A., Eds.; Designing functional foods; CRC Press: New York, pp 502-546.

Mene-Saffrane, L. & DellaPenna, D. (2010). Biosynthesis, regulation and functions of tocochromanols in plants. Plant Physiology and Biochemistry, 48, 301-309.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Menard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S. J. , Weitschies, W. & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food – an international consensus. Food & Function, 5, 1113-1124.

Mohnen, D. (2008). Pectin structure and biosynthesis. Current Opinion in Plant Biology, 11:266-77.

Morris, M. C., Evans, D. A., Tangney, C. C., Bienias, J. L., Wilson, R. S., Aggarwal, N. T. & Scherr, P. A. (2005). Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. American Journal of Clinical Nutrition 81, 508-514.

Munné-Bosch, S. & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. Critical Reviews in Plant Sciences, 21, 31-57. [CrossRef]

Nagy, S., (1980). Vitamin C contents of citrus fruit and their products: A review. Journal of Agricultural and Food Chemistry, 28: 8-18.

Nilsson, O. (1997). Rosa. In PH. Davis (Ed.). Flora of Turkey and the East Aegean Islands (pp. 106-128). (4th ed.). Edinburgh: Edinburgh University Press.

Ninomiya, K., Matsuda, H., Kubo, M., Morikawa, T., Nishida, N. & Yoshikawa, M. (2007). Potent anti-obese principle from *Rosa canina*: structural requirements and mode of action of transtiliroside. Bioorganic and Medicinal Chemistry Letters 17, 3059-3064.

Nováková, L., Solich, P. & Solichova, D. (2008). HPLC methods for simultaneousdetermination of ascorbic and dehydroascorbic acids. Trends in Analytical Chemistry, 27,942-958.

Okiei, W., Ogunlesi, M., Azeez. L., Obakachi, V., Osunsanmi, M. & Nkenchor, G. (2009). The voltametric and titrimetric determination of ascorbic acid levels in tropical fruits samples. International Journal of Electrochemistry, 4, 276-287.

Olsson, M. E., Gustavsson, K. E., Andersson, S., Nilsson, Å. & Duan, R. D. (2004). Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. Journal of Agricultural and Food Chemistry, 52, 7264-7271.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P. & Kwon, O. (2003). Vitamin C as an antioxidant: Evaluation of its role in disease prevention. Journal of the American College of Cardiology, 22, 18-35.

Parada, J. & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. J. Food Sci.72 (2), R21-32.pathway. Archives of Biochemistry and Biophysics, 483, 169-174.

Parr, A. J. & Bolwell, G. P. (2009). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of Science and Food Agriculture, 80: 985–1012

Péter, S., Friedel, A., Roos, F. F., Wyss, A., Eggersdorfer, M., Hoffmann, K. & Weber, P. (2016). A systematic review of global alpha-tocopherol status as assessed by nutritional intake levels and blood serum concentrations. International Journal for Vitamin and Nutrition Research**,** 14, 1-21.

Porat, R., Weiss, B., Cohen, L., Daus, A. & Aharoni, N., (2004). Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. Postharvest Biology and Technology, 33, 35-43.

Rao, A. V. (2002). Lycopene, tomatoes, and the prevention of coronary heart disease. Experimental Biology and Medicine, 227(10), 908-913.

Razungles, A., Oszmianski, J. & Sapis, J.-C. (1989). Determination of carotenoids in fruits of Rosa sp. (*Rosa canina* and *Rosa rugosa*) and of chokeberry (Aronia melanocarpa). Journal of Food Science, 54(3), 774-775.

Reboul, E., Richelle, M., Perrot, E., Desmoulins-Malezet, C., Pirisi, V. & Borel, P. (2006). Bioaccessibility of carotenoids and vitamin E from their main dietary sources. Journal of Agricultural and Food Chemistry, 54, 8749-8755.

Rodriguez-Amaya, D. B. (2001). A guide to carotenoid analysis in foods. ILSI Press, Washington, DC.

Rodríguez, J. C., Gómez, D., Pacetti, D., Núñez, O., Gagliardi, R., Frega, N., Ojeda, M. Loizzo, M., Tundis, R. & Lucci, P. (2016). Effects of the fruit ripening stage on antioxidant capacity, total phenolics, and polyphenolic composition of crude palm oil from interspecific hybrid Elaeis oleifera × Elaeis guineensis. Journal of Agricultural and Food Chemistry, 64, 852-859.

Rossnagel, K., Roll, S. & Wagner, A. (2007). Can patients with rheumatoid arthritis benefit from the herbal remedy rose-hip? Annals of the Rheumatic Diseases 66 (Suppl 2), 603.

Rousi, A. & H. Aaulin. (1977). Ascorbic acid contents in relation to ripeness in fruit of six H. rhamnoides clones form pyharanta, SW. Finland. Annales Agriculturae Fenniae, (16), 80-87.

Salvia-Trujillo, L., Qian, C., Martin-Belloso, O. & McClements, D. J. (2013). Modulating β-carotene bioaccessibility by controlling oil composition and concentration in edible nanoemulsions. Food Chemistry, 139, 878-884.

Schieber, A. & Carle, R. (2005). Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications. Trends in Food Science & Technology, 16, 416-422.

Schön, K. (1938). Studies on carotenoids V. gazaniaxanthin. Biochemical Journal, 32(9), 1566-1570.

Schweiggert, R. M., Kopec, R. E., Villalobos-Gutierrez, M. G., Högel, J., Quesada, S., Esquivel, P., Schwartz, S. J. & Carle, R. (2014). Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised cross-over study. British Journal of Nutrition. 111, 490-498.

Schweiggert, R. M., Mezger, D., Schimpf, F., Steingass, C. B. & Carle, R (2012). Influence of chromoplast morphology on carotenoid bioaccessibility of carrot, mango, papaya, and tomato. Food Chemistry, 135, 2736-2742.

Schweiggert, R. M., Steingass, C. B., Heller, A., Esquivel, P. & Carle, R. (2011). Characterization of chromoplasts and carotenoids of red- and yellow-fleshed papaya (Carica papaya L.). Planta, 234, 1031-1044.

Schweiggert, R. M. & Carle, R. (2017). Carotenoid deposition in plant and animal foods and its impact on bioavailability. Critical Reviews in Food Science and Nutrition. 57, 1807-1830.

Seema, P. (2012). Rose hips as complementary and alternative medicine: overview of the present status and prospects. Mediterranean Journal of Nutrition and Metabolism, 6(2), 89-97.

Siegel, D., Bolton, E. M., Burr, J. A.,Liebler, D. C. & Ross, D. (1997). The reduction of α-tocopherolquinone by human NAD(P)H: Quinone oxidoreductase: The role of α-tocopherolhydroquinone as a cellular antioxidant. Molecular Pharmacology, 52, 300-305.

Sila, D. N.,Van Buggenhout, S., Duvetter, T., Fraeye, I., De Roeck, A., Van Loey, A. & Hendrickx, M. (2009). Pectins in processed fruits and vegetables: Part II Structure function relationships. Comprehensive Reviews in Food Science and Food Safety, 8, 86-104.

Smirnoff, N. (2011). Vitamin C: the metabolism and functions of ascorbic acid inplants. In: Fabrice, R.b., Roland, D. (Eds.), Advances in Botanical Research. Academic Press, pp. 107-177.

Smirnoff, N., Conklin, P. L. & Loewus, F. (2001). Biosynthesis of ascorbic acid in plants A renaissance. Annual Review of Plant Physiology and Plant Molecular Biology, 52, 437- 467.

Solyom, K., Maier, C., Weiss, J., Cocero, M. J., Mato, R. B., Carle, R. & Schweiggert, R. (2014). Structure-response relationship of carotenoid bioaccessibility and antioxidant activity as affected by the hydroxylation and cyclization of their terminal end groups. Food Research International, 66, 107-114.

Staffan, C. A., Kimmo R., Eva, J. & Marie, E. O. (2008). Changes in tocopherols and carotenoids during ripening in Rose hips and sea buckthorn berries. Plants as Food and Medicine, 765

Stinco, C. M., Fernández-Vázquez, R., Escudero-Gilete, M. L., Heredia, F. J., Meléndez-Martínez, A. J. & Vicario, M. (2012) Effect of orange juice's processing on the color, particle size, and bioaccessibility of carotenoids. Journal of Agricultural and Food Chemistry, 60, 1447-1455.

Straight, R. C. & Spikes, J. D. (1985). Photosensitized oxidation of biomolecules. In Singlet O2; Frimer, A.A., Ed.; CRC Press: Boca Raton, FL, USA, Volume 4, pp. 91-143.

Thane, C. & Reddy, S. (1997). Processing of fruit and vegetables: effect on carotenoids. Nutrition & Food Science, 97(2), 58-65

Thibault, J. F., Renard, C. C., Axelos, M. V., Roger, P. & Crepeau, M. J. (1993). Studies of the length of homogalacturonic regions in pectins by acid-hydrolysis. Carbohydrate Research, 328, 271-286

Tsaniklidis, G., Delis, C., Nikoloudakis, N., Katinakis, P. & Aivalakis, G. (2014). Lowtemperature storage affects the ascorbics acid metabolism of cherry tomatofruits. Plant Physiology and Biochemistry, 84, 149-157.

Uggla, M., Gao, X. & Werlemark, G. (2003). Variation among and within dog rose taxa (Rosa sect. Caninae) in fruit weight, percentages of fruit flesh and dry matter, and vitamin C content. Acta Agriculturae Scandinavica, Section B- Soil & Plant Science, 53, 147-155.

Valente, A., Sanches-Silva, A., Albuquerque, T. G. & Costa, H. S. (2014). Development ofan orange juice in-house reference material and its application to guaranteethe quality of vitamin C determination in fruits juices and fruit pulps. Food Chemistry, 154, 71-77.

Valpuesta, V. & Botella, M. A. (2004). Biosynthesis of L-ascorbic acid in plants: newpathways for an old antioxidant. Trends in Plant Science, 9, 573-577.

Van het Hof, K. H., de Boer, B. C., Tijburg, L. B., Lucius, B. R., Zijp, I., West, C. E., Hautvast, J. G. & Weststrate, J. A. (2000). Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. Journal of Nutrition, 130 (5), 1189-1196.

Verlent, I., Hendrickx, M., Verbeyst, L. & Van Loey, A. (2007). Effect of temperature and pressure on the combined action of purified tomato pectin methylesterase and polygalacturonase in presence of pectin. Enzyme Microb. Enzyme and Microbial Technology, 40 (5), 1141-1146.

Wenzig, E. M., Widowitz, U., Kunert, O., Bauer, R. & Chrubasik, S. (2007). In vitro COX-1, COX-2 und 5-LOX inhibitory activity of rose hips (Rosae pseudofructus sine fructibus). Planta Medica 73, 844-845.

Werner, S. & Böhm, V. (2011). Bioaccessibility of carotenoids and vitamin E from pasta: evaluation of an in vitro digestion model. Journal of Agricultural and Food Chemistry, 59, 1163-1170.

Wicker, L., Kim, Y., Kim, M. J., Thirkield, B., Lin, Z. & Jung, J. (2014). Pectin as a bioactive polysaccharide - Extracting tailored function from less. Food Hydrocolloids, 42, 251-259.

Xu, H., Jiao, Q., Yuan, F. & Gao, Y. (2015). In vitro binding capacities and physicochemical properties of soluble fiber prepared by micro fluidization pre-treatment and cellulose hydrolysis of peach pomace. LWT - Journal of Food Science and Technology, 63, 677-684.

Yang, X. Y., Xie, J. X., Wang, F. F., Zhong, J., Liu, Y. Z., Li, G. H. & Peng, S. A. (2011). Comparison of ascorbate metabolism in fruits of two citrus species withobvious difference in ascorbate content in pulp. Journal of Plant Physiology, 168, 2196-2205.

Yildiz, O. & Alpaslan, M. (2012) Properties of rose hip marmalades. Food Technology and Biotechnology, 50, 98-106.

Yonekura, L. & Nagao, A. (2007). Intestinal absorption of dietary carotenoids. Molecular Nutrition & Food Research, 51, 107-115.

Youdim, K. A., Martin, A. & Joseph J. A. (2000). Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. Free Radical Biology and Medicine, 29 (1): 51- 60

Young, A. J. & Lowe, G. M. (2001) Antioxidant and prooxidant properties of carotenoids. Archives of Biochemistry and Biophysics, 385, 20-22.

Zadernowski, R., Naczk, M. & Amarowicz, R. (2003). Tocopherols in sea buckthorn (Hippophae rhamnoides L.) berry oil. Journal of the American Oil Chemists' Society, 80 (1), 55-58.

Zafra-Stone, S., Yasmin, T., Bagchi, M., Chatterjee, A., Vinson, J. A. & Bagchi, D. (2007). Berry anthocyanins as novel antioxidants in human health and disease prevention. Molecular Nutrition & Food Research, 51, 675-683.

Zhan, D., Janssen, P. & Mort, A. J. (1998). Scarcity or complete lack of single residues interspersed within the homogalacturonan region of citrus pectin. Carbohydrate Research 308, 373-80.

Zhan, L., Hu, J., Ai, Z., Pang, L., Li, Y. & Zhu, M. (2013). Light exposure during storagepreserving soluble sugar and L-ascorbic acid content of minimally processedromaine lettuce (Lactuca sativa L.var. longifolia). Food Chemistry, 136, 273-278.

Zhang, J. G., Luo, H. M., Huang, Q. J. Y. &. Shan, W. C. Y (2006). A comparative study on berry characteristics of large berry cultivars of sea buckthorn. Forest Research Beijing, 18, 643-650.

Zhang, R., Zhang, Z., Zhang, H., Decker, E. A. & McClements, D. J. (2015). Influence of emulsifier type on gastrointestinal fate of oil-in-water emulsions containing anionic dietary fiber (pectin). Food Hydrocolloids, 45, 175-185.

Zhong, L., Gustavsson, K. E., Oredsson, S., Gła, B., Yilmaz, J. L. & Olsson, M. E. (2016). Determination of free and esterified carotenoid composition in rosehip fruit by HPLC DAD-APCI+-MS. Food Chemistry, 210, 541-550.

Zhou, J. R., Gugger, E. T. & Erdman, J. W. (1996). The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of β- and α-carotene in the ferret model. Journal of the American College of Nutrition, 15, 84-91.

Scientific publications

Refereed journals, edited volumes and overviews

1. **Al-Yafeai, A.,** Böhm, V. (2015). Bioaccessibility of rosehip carotenoids - optimisation of an in vitro digestion model. Proc. Germ. Nutr. Soc. 20, 58. (abstract)

2. **Al-Yafeai, A.,** Böhm, V. (2015). Factors affecting bioaccessibility of rosehip carotenoids - *in vitro* results. Lebensmittelchemie 69, 105. (abstract)

3. **Al-Yafeai, A.,** Böhm, V. (2016). Carotenoids content, antioxidant capacity and vitamin C in *Rosa rugosa* and *Rosa canina* - Comparative study Proc. Germ. Nutr. Soc. 21, 50. (abstract)

4. **Al-Yafeai, A.,** Böhm, V. (2017). Analysis of carotenoids, vitamin E, vitamin C and antioxidant capacity in *R. rugosa* rosehips as affected by degree of ripeness. Lebensmittelchemie 7,123. (abstract)

5. **Al-Yafeai, A.,** Böhm, V. (2017). Bioactive compounds and antioxidant capacity of rosehips – comparative study of *R. rugosa* and *R. canina* Abstract-Book "18th International Symposium on Carotenoids", 09-14.07.2017, Lucerne, Switzerland, 178. (abstract)

6. **Al-Yafeai, A.,** Böhm, V. (2018). In vitro bioaccessibility of carotenoids and vitamin E in rosehip products and tomato paste as affected by pectin contents and food processing. Proc. Germ. Nutr. Soc. 24,33 (abstract)

7. **Al-Yafeai, A.,** Malarski, A., Böhm, V. (2018). Characterization of carotenoids and vitamin E in *R. rugosa* and *R. canina*: Comparative analysis. Food Chem. 242 (2018) 435-442.

8. **Al-Yafeai, A.,** Böhm, V. (2018). In vitro bioaccessibility of carotenoids and vitamin E in rosehip products and tomato paste as affected by pectin contents and food processing. J. Agric. Food Chem., 66, 3801-3809.

9. **Al-Yafeai, A**., Bellstedt, P. & Böhm, V. (2018). Bioactive compounds and antioxidant capacity of *R. rugosa* depending on degree of ripeness. Antioxidants 7, 134.

Presentations

1. **Al-Yafeai, A**., Böhm, V. (2015). Factors affecting bioaccessibility of rosehip carotenoids-*in vitro* results. Arbeitstagung des Regionalverbandes Süd-Ost der Lebensmittelchemischen Gesellschaft (LChG), Jena

2. **Al-Yafeai, A**., Böhm, V. (2017). Analysis of carotenoids, vitamin E, vitamin C and antioxidant capacity in *R. rugosa* rosehips as affected by degree of ripeness Arbeitstagung des Regionalverbandes Süd-Ost der Lebensmittelchemischen Gesellschaft (LChG), Halle/Saale

3. **Al-Yafeai, A.,** Böhm, V. (2018). In vitro bioaccessibility of carotenoids and vitamin E in rosehip products and tomato paste as affected by pectin contents and food processing. *55. Wissenschaftlicher Kongress der DGE,* Hohenheim.

Posters

1. **Al-Yafeai, A.,** Böhm, V. (2015). Bioaccessibility of rosehip carotenoids - optimisation of an in vitro digestion model. *52. Wissenschaftlicher Kongress der DGE, an der Martin-Luther-Universität, Halle-Wittenberg. 11.-13.03. 2015*

2. **Al-Yafeai, A.,** Böhm, V. (2016). Carotenoids content, antioxidant capacity and vitamin C in *Rosa rugosa* and *Rosa canina* - Comparative study *„53. Wissenschaftlicher Kongress der DGE, Fulda 02.-04.03.2016.*

3. **Al-Yafeai, A.,** Böhm, V. (2017). Bioactive compounds and antioxidant capacity of rosehips - comparative study of *R. rugosa* and *R. canina* Abstract-Book *18th International Symposium on Carotenoids, 09.-14.07.2017, Lucerne, Switzerland.*

Erklärungen

Hiermit erkläre ich eidesstattlich, dass:

- mir die geltende Promotionsordnung der Fakultät für Biowissenschaften der Friedrich-Schiller-Universität Jena bekannt ist.
- ich die vorliegende Dissertation selbst angefertigt habe und keine Textabschnitte eines Dritten oder eigener Prüfungsarbeiten ohne Kennzeichnung übernommen habe und alle benutzen Hilfsmittel, persönliche Mitteilungen und Quellen in der Arbeit angegeben habe.
- mich lediglich die in den Manuskripten angegebenen Personen bei der Auswahl und der Auswertung des Materials sowie bei der Erstellung der Manuskripte unterstützt haben.
- ich nicht die Hilfe eines Promotionsberaters in Anspruch genommen habe.
- Dritte weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorliegenden Dissertation stehen.
- ich diese Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe.
- ich weder die gleiche oder eine in wesentlichen Teilen ähnliche, noch eine andere Abhandlung bei einer anderen Hochschule als Dissertation eingereicht habe.

Jena, 26.October 2018

Ahlam Al-Yafeai

Acknowledgments

I would like to express my sincere gratitude to my supervisor PD Dr. habil. Volker Böhm for his constant support and assistance during the time of my Ph.D., I am greatly indebted to him for his great confidence and motivation he put in me since my first day in his work group. This work wouldn't have been possible without his guidance, involvement and encouragement, I have been benefited greatly from fruitful discussions with him which is put me in the right way and helped me to overcome many difficulties at work

Additionally, I would like to thank the staff members of the Bioactive Plant Products Research group for the great cooperation and support, especially Josephine Kschonsek, Angelika Malarski, Johanna Bößneck, Ernawita and Julia Schmidt

I greatly appreciate and acknowledge to the Society Freunde des Orients Jena e.V., which played a decisive role during my stay in Jena and especially it's President, Mohamed Yahya. I am truly grateful to him for guidance, advice, and help for me

Finally, I would like to express my appreciation to the people who mean the world for me, my parents, (Baba Hamood and Mama Amna) my brothers and sisters for their immeasurable love and care, sacrifices, unceasing encouragement, and support which helped me stay strong during these difficult years and inspired me to finish this work.

At last I wish to thank many other people whose names are not mentioned here but this does not mean that I have forgotten their help.

Ahlam Al-Yafeai