Black carrot polyphenols: Effect of processing, storage and digestion – an overview

Senem Kamiloglu[1,2], John Van Camp[2], & Esra Capanoglu[1]*

[1] Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, Maslak, 34469, Istanbul, Turkey

[2] Laboratory of Food Chemistry and Human Nutrition (nutriFOODchem), Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

*Corresponding author.

Tel: +90 212 2857340

Fax: +90 212 2857333

E-mail: capanogl@itu.edu.tr

Abstract

Black carrots represent a valuable source of polyphenols, in particular anthocyanins and phenolic acids, and has attracted the attention of the scientific community especially due to the unique profile of anthocyanin compounds, which are well distinguished for their role in health promotion and prevention of chronic diseases. Black carrots are often not consumed as such, instead they are processed into other products. In general, processed products of black carrot are stored for long term and the polyphenols are susceptible to degradation during storage. In addition, it is also important to determine how the digestion process affects polyphenols as this will, in turn, affect their bioavailability. Accordingly, the potential health-promoting effects of black carrot polyphenols depend on their processing history and their stability during storage as well as their absorption in the gastrointestinal tract. In this perspective, this review provides an overview of the findings on the effects of processing, storage and digestion on black carrot polyphenols.

Keywords: anthocyanins; bioavailability; *Daucus carota* L.; food preservation; phenolic acids

Abbreviations

ACN: anthocyanin; HCA: hydroxycinnamic acid; LDH: lactate dehydrogenase; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PA: phenolic acids; ROS: reactive oxygen species; SHIME: simulator of the human intestinal microbial ecosystem; TFC: total flavonoid content; TPC: total phenolic content

Introduction

Carrots (*Daucus carota* L.), a member of Apiaceae (formerly Umbelliferae) family (Erten et al. 2008), constitute a valuable source of health-promoting ingredients and thus are important in human nutrition, with an annual worldwide production exceeding 35.6 million of tons (Carvalho et al. 2014). Cultivated carrots can be subdivided into two main groups, the western or carotene carrot (*Daucus carota* ssp. *sativus* var. *sativus*) and the eastern or anthocyanin carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) (Kammerer et al. 2004b). Although, orange carrot varieties account for the majority of this crop, black or purple carrots are thought to be much older than orange carrot varieties. In fact, black carrots, originating from oriental countries such as Turkey, Afghanistan, Egypt, Pakistan, India and the Far East, have been cultivated for at least 3000 years (Schwarz et al. 2004). In Europe, black carrots have been cultivated from the Middle Ages to the 18th century until orange varieties became predominant (Kammerer et al. 2003). Nowadays, although orange carrots are more common, consumption of black carrots is also increasing (Algarra et al. 2014).

Black carrots have an attractive bluish-purple color with high levels of anthocyanins and can serve as a natural food colorant due to their high heat, light, and pH stability (Montilla et al. 2011). Providing an excellent bright strawberry red shade at acidic pH values, black carrots are being used as a nutraceutical/functional ingredient in various food matrices including fruit juices and nectars, soft drinks, preserves, jellies and confectionary (Khandare et al. 2011; Murali et al. 2015). Apart from their colorant properties, black carrots also attracted attention due to their substantial nutrient content. Based on the information provided in Turkish food composition database (TurKomp, http://www.turkomp.gov.tr), black carrot roots contain approximately 88% water, 1% protein, 8% carbohydrate, 0.14% fat, and 2.5% fiber. Black carrots are also significant

sources of certain minerals and vitamins. On the other hand, unlike other carrots with different root colors they do not contain carotenoids (Table 1). Instead, black carrots drew attention due to the presence of polyphenols.

Like many fresh fruits and vegetables, black carrots are subjected to some form of processing due to their seasonal and perishable nature. In general, these processed products are stored for long term and the polyphenols are susceptible to degradation during storage. In addition, it is also important to determine how the digestion process affects polyphenols as this will affect their bioavailability. Accordingly, the potential health-promoting effects of black carrot polyphenols depend on their processing history and their stability during storage as well as their absorption in the gastrointestinal tract. In this perspective, this review provides an overview of the findings on the effects of processing, storage and digestion on black carrot polyphenols.

Characterization of black carrot polyphenols

As anthocyanins and phenolic acids are the predominant polyphenols present in black carrots (Netzel et al. 2007), in the following sections these compounds will be discussed in detail.

The major anthocyanins identified in black carrots were cyanidin-based: cyanidin-3-xylosyl-glucosyl-galactoside, cyanidin-3-xylosyl-galactoside and the sinapic, ferulic and coumaric acids derivatives of cyanidin 3-xylosyl-glucosyl-galactoside (Wallace and Giusti 2008; Montilla et al. 2011; Garcia-Herrera et al. 2016). Compounds with an acylated structure constitute more than half of total anthocyanins contained in black carrots, whereas the predominant anthocyanin corresponded to cyanidin-3-xylosyl-feruloyl-glucosyl-galactoside. Trace amount of peonidin and pelargondin glycosides, which correspond to peonidin-3-xylosyl-glucosyl-galactoside, ferulic acid derivative of

pelargonidin-3-xylosyl-glucosyl-galactoside and ferulic acid derivative of peonidin-3-xylosyl-glucosyl-galactoside, have also been identified in black carrots (Figure 1). The identification of anthocyanins was made using HPLC based on the UV–visible features, mass spectrometry and fragmentation pattern (Kammerer et al. 2003; Algarra et al. 2014). The increased number of sugar moieties in the anthocyanin molecule and the acylating moieties provide protection to the aglycone from the hydrophilic attack of water, and contribute to intramolecular co-pigmentation effect, which results in increased heat- and light-stability and extended shelf-life of the pigment (Day et al. 2009). Total anthocyanin content in the roots of black carrots can vary widely between cultivars and even within a cultivar based on the degree of root coloring (Lazcano et al. 2001; Kammerer et al. 2004b). Black carrots are reported to contain up to 350 mg anthocyanins per 100 g fresh weight (fw). For comparison, total anthocyanin concentration is approximately 113 mg/100 g fw in blueberries, 117 mg/100 g fw in cherries, and 48 mg/100 g fw in raspberries (Arscott and Tanumihardjo 2010). According to a report, anthocyanins accounted for about 42% of the total phenolics present in purple carrot extracts (Wallace and Giusti 2008). Similarly, in another study, the level of anthocyanins corresponded to 50% of the total phenolic content of black carrots for Antonina variety (Algarra et al. 2014). Several studies are carried out to optimize the extraction of black carrot anthocyanins under different conditions (Gizir et al. 2008; Turker and Erdogdu 2006; Guldiken et al. 2016). Shortening the extraction time by low pH and high temperature is found to be feasible for an industrial process (Turker and Erdogdu 2006).

Besides anthocyanins as the major polyphenols, black carrots also contain significant amounts of phenolic acids. The majority of the phenolic acids identified in black carrots were hydroxycinnamic acid derivatives. Chlorogenic acid (5-*O*-caffeoylquinic acid), an ester of caffeic and quinic acid (Figure 2), was found to be the

predominant compound, amounting to 657 mg/kg in the roots of black carrot. Neochlorogenic acid (3-*O*-caffeoylquinic acid) and cryptocholorogenic acid (4-*O*-caffeoylquinic acid) are among the other major hydroxycinnamic acids identified in black carrots. In addition, significant amounts of caffeic acid and ferulic acid are also detected in black carrots. Similar to anthocyanins, HPLC coupled to mass spectrometry was helpful for characterization of phenolic acids (Kammerer et al. 2004a). The presence of phenolic acids contributes significantly to the stability of black carrot polyphenolic complex through intermolecular or intramolecular co-pigmentation effect (Day et al. 2009). Studies showed that black or purple carrots contained higher phenolic content than roots of other colors (Grassmann et al. 2007; Sun et al. 2009; Leja et al. 2013; Koley et al. 2014).

In many studies in the literature an organic solvent, e.g., methanol or ethanol, is used to extract polyphenols from black carrots. It has been shown that under optimized conditions, subcritical water might be a good substitute to organic solvents such as methanol and ethanol to extract polyphenols from plant sources (Singh and Saldana 2011). Hence, for the sake of both the environment and human health, in future studies, "green solvent" aspects should be taken into account.

Health effects of black carrot polyphenols

In vitro studies

Several studies showed that black carrot polyphenols possess *in vitro* bioactive properties including antioxidant, anti-proliferative, anti-diabetic and anti-inflammatory activities. As an example, crude phenolic extract from black carrot concentrate showed strong radical scavenging activity (≈125 μM Trolox Eq./g dw), equivalent to or higher than that of other selected fresh fruits such as cherry (≈55 μM Trolox Eq./g dw), or

berries: strawberry, raspberry and blueberry (85−120 μM Trolox Eq./g dw), that are considered to be the health-promoting fruits due to high polyphenol content (Day et al. 2009). Furthermore, purified anthocyanin-rich polyphenol extract from black carrot inhibited proliferation of both HT-29 colorectal adenocarcinoma and HL-60 promyelocytic leukaemia cells in a dose-dependent manner, and at the concentration of 2.0 mg/mL a suppression of about 80% was observed (Netzel et al. 2007). Similarly, polyphenols from fermented black carrot juice inhibited the growth of intestinal Caco-2 cells in a dose-dependent manner, decreasing to 59.3% at the concentration of 6.4 mg/mL (Ekinci et al. 2016). Olejnik et al. (2016) indicated that digested purple carrot extract is capable of NCM460 colonic cells' protection against the adverse effects of oxidative stress including ROS production and DNA damage, with 1 mg/mL extract showing the ROS clearance of 18.4% and 20.7% reduction in oxidative DNA damage. A recent study pointed out that Deep Purple carrot extract exhibited an inhibitory effect on α-amylase and α-glucosidase activity in a dose-dependent manner (50% reduction at concentrations of 7.97 mg/mL and 5.04 mg/mL, respectively) and on intestinal glucose uptake in intestinal Caco-2 cells (decreasing to approximately 90% at concentration of 0.1 mg/mL) (Esatbeyoglu et al. 2016). In addition, in our latest work we demonstrated that the black carrot polyphenols transported through intestinal Caco-2 cell monolayer (0.8−5.2 μM anthocyanins and 1.2−2.0 μM phenolic acids) were able to down-regulate the secretion of pro-inflammatory markers from 120−203% to 34−144% in endothelial EA.hy926 cells (Kamiloglu et al. 2017a). Besides polyphenols, black carrots also contain polyacetylene compounds such as falcarindiol, falcarindiol 3-acetate and falcarinol (Christensen and Kreutzmann 2007). Falcarindiol from purple carrots (10 μM) were reported to reduce the nitric oxide production in macrophage cells by as much as 65% without cytotoxicity.

Considering that it has been suggested that polyacetylenes could also be responsible for anti-inflammatory activity of purple carrots (Metzger et al. 2008).

In many studies mentioned above, extremely high concentrations of black carrot extracts are used to detect a biological response. It is well known from the literature that *in vitro* effects observed at extract concentrations >50 µg/mL or compound concentrations >5 µM are questionable (Gertsch 2009) and higher concentrations may result in markedly increased incidence of false-positives and non-specific cell toxicity. Cytotoxicity on host cells is an important criterion for assessing the selectivity of the observed biological activities and should always be included in parallel (Cos et al. 2006). In all the studies in which biological activities of black carrot polyphenols are discussed, toxicity tests including MTT (Netzel et al. 2007; Olejnik et al. 2016; Kamiloglu et al. 2017a), MTS (Ekinci et al. 2016) and LDH (Esatbeyoglu et al. 2016) are used to assess the cytotoxic dose of black carrot extracts. In these assays, cell viability is expressed as percentage relative to the untreated cells (control). The results of the studies determining the cytotoxicity of black carrot polyphenols on various cell lines is given in Table 2. As seen in the table, the cytotoxic dose of black carrot extracts differs depending on the type of activity studied. This indicates that all the different biological activities reported in the literature cannot be present at the same time as they are all dose dependent. In this case the lowest concentration, which shows an activity with the highest effect, should be selected.

Considering the above, a number of quality standards need to be set at the level of extract processing and primary evaluation in *in vitro* screening models. These requirements include: (i) use of reference strains or fully characterized clinical isolates, (ii) *in vitro* models on the whole organism and if possible cell-based, (iii) evaluation of selectivity by parallel cytotoxicity testing, (iv) adequately broad dose range, enabling

dose–response curves, (v) stringent endpoint criteria with IC_{50}-values generally below 100 µg/mL for extracts, (vi) proper preparation, storage and in-test processing of extracts, (vii) inclusion of appropriate controls in each *in vitro* test replicate and (viii) follow-up of *in vitro* activity in matching animal models (Cos et al. 2006).

In vivo studies

Anthocyanins and phenolic acids from black carrots were reported to be effective at reversing inflammation and metabolic alterations in animal models, potentially through inhibition of inflammatory pathways. For instance, purple carrot juice (5% of the diet) reduced abdominal obesity, systolic blood pressure, plasma lipids, hepatic steatosis, cardiac fibrosis, and inflammation along with improved glucose tolerance in high-carbohydrate, high-fat diet-fed rats (Poudyal et al. 2010). The authors reported that it is likely that the anthocyanins were responsible for the observed effects. Similarly, black carrot extracts fermented with *Aspergillus oryzae* prevented the impairment of energy, lipid and glucose metabolism in estrogen-deficient rats (Park et al. 2015). In another study performed by the same group, black carrot extracts fermented with *Aspergillus oryzae* and *Lactobacillus plantarum* showed potent efficacy for improving cognitive function by preventing hippocampal insulin resistance in type 2 diabetic rats with dementia (Park et al. 2016). On the other hand, a human trial, which investigated the effects of dried purple carrot on body mass, body composition, blood pressure, lipids, inflammatory markers, liver function tests, and appetite in 16 volunteers with normal lipid and inflammatory markers, concluded that there was no evidence that 118.5 mg/day of anthocyanins and 259.2 mg/day of phenolic acids for 4 weeks resulted in statistically significant changes in body mass, body composition, appetite, dietary intake, low density lipoprotein, total cholesterol, blood pressure, or C-reactive protein in obese participants at the dose and length of intervention (Wright et al. 2013).

Nowadays, the use of metabolomics in polyphenol research has attracted great attention. In particular, this technique is now being used to study the inter-individual variations in the metabolism of dietary polyphenols. Experts in polyphenol research propose that metabolomics can contribute to a better understanding of the complex interactions between polyphenol intake and human health. Considering that, we expect to see more nutrimetabolomics research, especially on inter-individual differences, in the near future.

The EFSA guidance on scientific requirements for health claims related to gut and immune function (EFSA Panel on Dietetic Products Nutrition and Allergies 2011) states that chronic inflammation is associated with the development of a number of diseases, and that altering levels of markers of inflammation might indicate a beneficial physiological effect in the context of a reduction of disease risk claim, if it can be demonstrated that altering the levels of inflammatory markers is accompanied by a reduced incidence of a disease for a specific dietary intervention. Currently, the EU health claim register does not contain any authorized or non-authorized health claims that specifically address the health benefit area of suppression or control of low-grade inflammation. The lack of health claims is probably due to the fact that, although many biologically relevant mechanisms have been established to explain inflammation–disease associations, no single biomarker or group of biomarkers of inflammation has yet been strongly demonstrated to be sufficiently predictive of future disease. A suggested strategy for building an EFSA health claim on this topic comprises (i) a description of product composition; (ii) a well grounded selection of the target population; (iii) the selection of a clinically relevant composite biomarker panel representing inflammation as well as the selected health benefit endpoints; and (iv) several sufficiently powered and well-controlled human studies assessing the effect of the test material on the relevant

biomarkers in the relevant target population (Minihane et al. 2015). Overall, in the future to build health claims on black carrot polyphenols for improving inflammation, researchers should first focus on the need for well-validated biologically relevant markers that reflect the inflammatory state.

The potential health-promoting properties of black carrot polyphenols indicated above will depend on their stability during processing, storage and digestion. In the following sections these conditions are discussed in detail.

Effect of food processing on black carrot polyphenols

The need for food processing arises from various reasons: (i) to prolong the shelf-life of fresh product; (ii) to provide products out of season; (iii) to produce convenient products for home consumption; (iv) to supply novel food products with alternative flavor, texture and color; or (v) to provide improved nutritional properties. Effect of food processing on polyphenol content of fruits and vegetables has been studied extensively (Nicoli et al. 1999; Kalt 2005; Kamiloglu et al. 2016b; Nayak et al. 2015; Rothwell et al. 2015). Although the impact of process differs widely according to the type of treatment, the conditions of the process applied, and also the variety, source or cultivation history of the fruit/vegetable used, for anthocyanins generally adverse effects have been reported (Patras et al. 2010). Hence, attention should be given to minimize the detrimental effects of food processing and the subsequent storage conditions used.

The studies on the effect of processing on black carrot polyphenols are presented in Table 3. Heat treatment caused degradation of black carrot anthocyanins, which was more rapid at increased heating temperatures (Ersus and Yurdagel 2007; Kirca et al. 2007; Murali et al. 2015). On the other hand, increased pH values caused only a small overall increase in anthocyanin degradation compared to values at low pHs (Iliopoulou et al.

2015). Thermal treatment resulted in a first-order reaction model of degradation for the monomeric anthocyanins in black carrots (Kirca et al. 2007; Reyes and Cisneros-Zevallos 2007). Acylated anthocyanins were more resistant to thermal degradation compared to non-acylated anthocyanins. Upon severe heating, acylated anthocyanins are cleaved into the corresponding acyl-glycosides and the aglycones the latter being readily transformed into the intermediate chalcone, which is instantly cleaved into protocatechuic acid and phloroglucinaldehyde (Sadilova et al. 2007). In order to increase the stability of black carrot anthocyanins, microencapsulation and co-pigmentation methods have been employed. Studies showed that maltodextrin 20DE as the carrier material has proven to be better in retaining maximum anthocyanins (Ersus and Yurdagel 2007; Murali et al. 2015) and anthocyanins were better entrapped in whey protein hydrogels compared to other phenolic compounds from black carrots (Ersus Bilek et al. 2017). Combination of black carrot extracts with chlorogenic acid as co-pigments barely improved the anthocyanin stability unless the concentrations of co-pigments exceeded those of their natural source (Gras et al. 2016). Sugar and ascorbic acid addition also resulted in a slight stabilizing effect of black carrot anthocyanins (Sadilova et al. 2009). A study comparing the effect of different drying methods on anthocyanin content of various purple carrot roots revealed that for Deep Purple cultivar, the most advantageous drying method was microwave-convection drying, whereas convection drying caused the highest degradation. On the other hand, for roots of Purple Haze cultivar the most advantageous method was freeze-drying, while infrared convective drying was proved to be least advantageous (Witrowa-Rajchert et al. 2009). Pre-press maceration treatment with cell wall degrading enzyme pectinase (*Aspergillus niger* Teigh) significantly improved the total phenolic, flavonoid and anthocyanin content of black carrot juice (Khandare et al. 2011). Similarly, in other studies while depectinization resulted in increased content of

anthocyanins (Turkyilmaz et al. 2012), total phenolics and hydroxycinnamic acids (Dereli et al. 2015), gelatine–kieselsol treatment led to reductions in polyphenols. Bentonite treatment and pasteurization resulted in varying effects, causing both reductions and enhancements in polyphenol content of black carrot juice (Turkyilmaz et al. 2012; Dereli et al. 2015). Processing black carrots into concentrate led to reductions in phenolics, flavonoids and anthocyanins (Suzme et al. 2014; Ersus Bilek et al. 2017). Differences observed in degradation mechanisms of anthocyanins under solid and aqueous conditions. In particular, at high pHs anthocyanins at solid state were reported to be significantly more stable compared to aqueous conditions. Moreover, the stability of the acylated anthocyanins was markedly higher in the solid state than in the liquid form (Iliopoulou et al. 2015). In our study, we observed that jam and marmalade processing significantly decreased total phenolics, anthocyanins and phenolic acids, whereas use of sweetener instead of sugar in jams and marmalades did not lead to a statistically significant difference in polyphenol content (Kamiloglu et al. 2015a; b). In a recent study, fermentation of black carrots with *Saccharomyces cerevisiae* to produce an alcoholic beverage resulted in increased total phenolic content, while the changes in anthocyanins was not significant (Kocher et al. 2016).

Although in general food processing decreased the anthocyanin content in black carrots, stability of anthocyanins to pH and thermal degradation was reported to be higher compared to anthocyanins from grape and purple-flesh potatoes (Reyes and Cisneros-Zevallos 2007). Similarly, black carrot juice anthocyanins exhibited higher retention towards thermal treatment compared to elderberry and strawberry juices. Better stability may be attributed to the high ratio of acylated anthocyanins in black carrot samples compared to the non-acylated pigments (Sadilova et al. 2009).

The fast growing food industry generates by-products obtained during procesing. These by-products are usually disposed both in environmental and economical terms. They are often discarded as waste or, in the best case valorized in low-value applications such as animal feed and bio-energy applications. However, these by-products have the potential to be used as relatively cheap but valuable resources for bioactive compounds. In many cases by-products contain the same valuable constituents as the starting products and possibly yet uncharacterized active compounds that could lead to higher value applications. Considering that, in one of our recent study (Kamiloglu et al. 2016a), we analyzed the polyphenol content of black carrot by-products, i.e., peel and pomace. The results of our study revealed that individual anthocyanin and phenolic acid contents of pomace was found to be higher than that of black carrots. This observation may be explained by the release of bound compounds with the breakdown of cellular constituents (i.e., cellulose and cellulose-pectin composites) a consequence of applied temperature and enzyme treatment during black carrot juice processing (Kamiloglu et al. 2016a). Considering the content of polyphenols retained in black carrot pomace, next we proposed that this by-product can be considered as a potential food ingredient and that it can be used to improve the functional properties of cakes. Accordingly, we enriched cake flour with black carrot pomace powder at levels of 50 g/kg, 100 g/kg and 150 g/kg, and compared the changes in total phenolics with the control cake containing no pomace. Results showed that addition of pomace caused a dose-dependent increase in total phenolic content (Kamiloglu et al. 2017b). Studies on the by-products of black carrot processing are very limited. However, we believe that these by-products deserve extra attention as they represent a potentially valuable source of functional ingredients for the food industry providing not only a high content of polyphenols but also a considerable amount of dietary fiber.

Development of a functional food product requires identification of functional compounds and assessment of their physiological effects, product formulation and development of a suitable food matrix, clinical trials on product efficacy in order to get approval for the health claims and market and public acceptance (Siro et al. 2008). As indicated above, we recently proposed that black carrot pomace can be used as a functional food ingredient due its high anthocyanin content and we evaluated the possible effects of the cake matrix (Kamiloglu et al. 2017b). However, before introducing this product to clinical trials, there is another important issue that needs to be resolved, which is the organoleptic properties of the product. Once the sensorial properties of this product are approved, then a double-blind, randomized, placebo-controlled design should be performed. One of the important factors in clinical trials is to decide the dose of administration. According to the scientific opinion of EFSA on the use of anthocyanins as a food additive (EFSA Panel on Food Additives and Nutrient Sources added to Food 2013), the currently available toxicological database is inadequate to establish a numerical ADI for anthocyanins. Therefore, further research on toxicological data of anthocyanins is necessary. Finally, although often ignored, the consumer acceptance of such product also needs to be investigated.

Effect of storage on black carrot polyphenols

Studies investigating the stability of black carrot polyphenols during storage are presented in Table 4. In general, storage at higher temperatures resulted in faster anthocyanin degradation as compared to storage at lower temperatures (Turker et al. 2004; Ersus and Yurdagel 2007; Kirca et al. 2007; Ozen et al. 2011; Turkyilmaz and Ozkan 2012; Kamiloglu et al. 2015a). In fact, anthocyanin content of black carrots did not change significantly during storage at cold temperatures (Lee et al. 2011; Zozio et al.

2011). Acylated antocyanins were significantly more stable than the non-acylated anthocyanins at all storage temperatures (Turker et al. 2004; Turkyilmaz and Ozkan 2012; Kamiloglu et al. 2015a). Pasteurization and sorbate treatment showed no significant positive or negative effects on total anthocyanin content or on the anthocyanin profile with respect to control at any storage temperature ($p < 0.05$) (Turker et al. 2004). The degradation of anthocyanins in purple carrots was particularly significant in the modified atmosphere packaging treatment of 95% O_2 and 5% CO_2 ($p < 0.05$) (Alasalvar et al. 2005). On the other hand, yogurt matrices containing higher fat contents showed increased stability of anthocyanins during two months of storage (Wallace and Giusti 2008). Several studies confirmed that the degradation of black carrot anthocyanins during storage at high temperatures fitted a first-order reaction model (Kirca et al. 2007; Ozen et al. 2011; Turkyilmaz and Ozkan 2012; Zozio et al. 2011; Wallace and Giusti 2008). Although thermal storage reduced the amount of black carrot anthocyanins, the rate of degradation has been shown to be slower than açai and blackberry anthocyanins (Zozio et al. 2011).

Effect of digestion on black carrot polyphenols

In vitro studies

Although *in vivo* trials are still being considered as the "gold standard" for addressing diet-related issues, *in vitro* models have the advantages of being more rapid and less expensive, and having more standardized operation conditions and no ethical restrictions. Considering that we have studied the fate of black carrot polyphenols after digestion using three different *in vitro* models (Kamiloglu et al. 2015a; Kamiloglu et al. 2016a; Kamiloglu et al. 2017b). Regardless of the *in vitro* digestion model used, the amount of black carrot polyphenols released during digestion was lower than what was

388 initially present in undigested samples. Another recent *in vitro* digestion study on
389 fermented black carrot juice also reported lower release of phenolics (Degirmencioglu et
390 al. 2016). These observations may be related to the fact that polyphenols, in particular
391 anthocyanins may be metabolized to some non-colored forms (i.e. chalcones), oxidized,
392 or degraded into other compounds (phenolic acids and aldehydes). In addition, a large
393 amount (65%) of polyphenols from black carrots, i.e., anthocyanins and phenolic acids,
394 was reported to be bound to plant cell walls and overwhelmingly remain bound after *in*
395 *vitro* gastric and small intestinal digestion (Padayachee et al. 2013). Another finding in
396 common for all digestion models was that the availability of non-acylated anthocyanins
397 was higher than acylated anthocyanins, which was also in line with *in vivo* studies on
398 black carrot polyphenols (Kurilich et al. 2005; Netzel et al. 2007; Charron et al. 2009;
399 Novotny et al. 2012).

400 One of the major problems to be resolved considering the *in vitro* digestion
401 models is that the majority of these models do not consider the fact that polyphenols may
402 be transformed by the gut microbiota (to ring fission products) and by intestinal cells (to
403 conjugates such as sulfated, glucuronidated and methylated forms). Although we tried to
404 employ these conditions by using SHIME suspension (Kamiloglu et al. 2016a) and
405 intestinal Caco-2 cells (Kamiloglu et al. 2017b), we could not have the chance to identify
406 all the gut and intestinal metabolites of black carrot. In addition, polyphenol-microbiota
407 interactions are complex and subject to interindividual variability, leading to different
408 polyphenol-metabolizing phenotypes or metabotypes (Bolca et al. 2013).
409 Characterization of these metabotypes and evaluation of microbial intermediates and end
410 products is crucial to maximize the overall health benefits from polyphenols. Therefore,
411 in future studies it would be interesting to pay attention to these metabolites of black
412 carrot, which may be responsible from the biological activities.

413 Some studies suggest that the rapid appearance of anthocyanins in plasma after
414 consumption could result from their absorption through the gastric wall. In fact, research
415 conducted with rats confirmed that anthocyanins are rapidly absorbed from the stomach
416 (Felgines et al. 2006; Talavera et al. 2003; Passamonti et al. 2003). An alternative to
417 animal studies for predicting stomach absorption of anthocyanins could be the use of a
418 gastric cell culture model. A critical feature of such a model is that it has to work in the
419 presence of a reduced pH. In a recent work (Fernandes et al. 2012) moderately
420 differentiated adenocarcinoma stomach cells (MKN-28) were used as a gastric barrier
421 model to study the absorption of anthocyanins at different pH conditions (pH of 3.0 and
422 5.0). The authors concluded that anthocyanin standards, namely delphinidin-3-glucoside,
423 cyanidin-3-glucoside and malvidin-3-glucoside could cross the gastric epithelium in a
424 time dependent manner with no statistical differences in their transport efficiency
425 according to the pH. In the future, this model can be improved by co-culturing the gastric
426 MKN-28 cell line with the mucin secreting cell line HT29-MTX to better simulate the
427 stomach conditions, which then can be used to study the gastric absorption of black carrot
428 anthocyanins.
429 *In vivo* studies
430 The majority of studies on the bioavailability of black carrot polyphenols have
431 focused particularly on anthocyanins. Direct evidence on bioavailability of black carrot
432 anthocyanins has been achieved by measuring the concentration of the compounds in
433 biological fluids, mainly plasma and urine, after ingestion of black carrots in different
434 matrices (e.g. raw, cooked, juice etc.). Research conducted on humans (Table 5) has
435 shown that most of the black carrot anthocyanins were present in plasma and urine in
436 intact form. Recovery of non-acylated anthocyanins was significantly higher than that of
437 acylated anthocyanins (Kurilich et al., 2005; Netzel et al., 2007; Charron et al., 2009;

Novotny, Clevidence, & Kurilich, 2012). A kinetic study reported that the acylated anthocyanins exhibited a shorter half-life for gastrointestinal absorption than the non-acylated anthocyanins (Novotny et al., 2012). In a clinical feeding study that used both raw and cooked purple carrots as anthocyanin source, it has been shown that cooking increases the recovery of non-acylated anthocyanins, but not for acylated anthocyanins (Kurilich et al., 2005). Furthermore, increased dose of administration resulted in reduced recovery of both acylated and nonacylated anthocyanins (Kurilich et al., 2005; Charron et al., 2009). Only a single study was able to identify metabolites, namely cyanidin-monoglucuronide and cyanidin-monosulfate, in biological fluids. However, the authors noted that the concentrations of these metabolites were near or below the limit of quantification (Netzel et al., 2007).

Encapsulation technology can be used to enhance the bioavailability of polyphenols or to achieve controlled release of these compounds during digestion. Spray drying, coacervation, liposome entrapment, inclusion complexation, cocrystallization, nanoencapsulation, freeze drying, yeast encapsulation and nanoemulsion are some of the current technologies that are used to encapsulate polyphenols (Fang and Bhandari 2010). Although there are few reports in the literature that studied the encapsulation of black carrot polyphenols, in particular anthocyanins (Ersus and Yurdagel 2007; Murali et al. 2015; Ersus Bilek et al. 2017), the effect of this processing technique on the human body is not established yet. Therefore, in the future it would be interesting to do some research on this topic.

Conclusions and recommendations for future research

In this review, the current knowledge about effects of processing, storage and digestion on black carrot polyphenols is presented. Studies have shown that the

polyphenol content of black carrots decrease after processing, storage and digestion. To reduce these detrimental effects, implementations such as reducing the processing and storage temperatures may be applied. In addition to the research performed in the literature, new strategies need to be addressed to gain additional information about black carrot polyphenols. Below, a number of important points are highlighted as to how, in the future, the researchers might approach this topic differently, in order to maximize the knowledge gained from this overview.

- Metabolomics can be used to study the inter-individual variations in the metabolism of black carrot polyphenols.
- Encapsulation technology can be used to enhance the bioavailability of black carrot polyphenols or to achieve controlled release of these compounds during digestion.
- Gastric cell line model that simulate the stomach conditions can be used to study the gastric absorption of black carrot anthocyanins.
- Gut metabolites of black carrot, which may be responsible from the biological activities, should be taken into account.
- To use black carrot by-products for enrichment of food products, a double-blind, randomized, placebo-controlled design should be performed along with consumer acceptance survey.
- Finally, to build health claims on black carrot polyphenols, researchers should focus on the need for well-validated biologically relevant markers.

References

Alasalvar C, Al-Farsi M, Quantick PC et al (2005) Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids,

phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. Food Chem 89:69-76

Algarra M, Fernandes A, Mateus N et al (2014) Anthocyanin profile and antioxidant capacity of black carrots (Daucus carota L. ssp. sativus var. atrorubens Alef.) from Cuevas Bajas, Spain. Journal of Food Composition and Analysis 33:71-76

Arscott SA, Tanumihardjo SA (2010) Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. Comprehensive Reviews in Food Science and Food Safety 9:223-239

Bolca S, Van de Wiele T, Possemiers S (2013) Gut metabotypes govern health effects of dietary polyphenols. Current opinion in biotechnology 24:220-225

Carvalho D, Dominguez A, Neto DO et al (2014) Combination of sowing date with deficit irrigation for improving the profitability of carrot in a tropical environment (Brazil). Scientia Horticulturae 179:112-121

Charron CS, Kurilich AC, Clevidence BA et al (2009) Bioavailability of anthocyanins from purple carrot juice: Effects of acylation and plant matrix. Journal of Agricultural and Food Chemistry 57:1226-1230

Christensen LP, Kreutzmann S (2007) Determination of polyacetylenes in carrot roots (Daucus carota L.) by high-performance liquid chromatography coupled with diode array detection. Journal of Separation Science 30:483-490

Cos P, Vlietinck AJ, Berghe DV et al (2006) Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. Journal of Ethnopharmacology 106:290-302

Day L, Seymour RB, Pitts KF et al (2009) Incorporation of functional ingredients into foods. Trends in Food Science & Technology 20:388-395

511 Degirmencioglu N, Gurbuz O, Sahan Y (2016) The monitoring, via an in vitro digestion
512 system, of the bioactive content of vegetable juice fermented with Saccharomyces
513 cerevisiae and Saccharomyces boulardii. J Food Process Pres 40:798–811
514 Dereli U, Turkyilmaz M, Yemis O et al (2015) Effects of clarification and pasteurization
515 on the phenolics, antioxidant capacity, color density and polymeric color of black
516 carrot (Daucus carota L.) juice. Journal of Food Biochemistry 39:528-537
517 EFSA Panel on Dietetic Products Nutrition, Allergies (2011) Guidance on the scientific
518 requirements for health claims related to gut and immune function. EFSA Journal
519 9:1984-1995
520 EFSA Panel on Food Additives and Nutrient Sources added to Food (2013) Scientific
521 Opinion on the re-evaluation of anthocyanins (E 163) as a food additive. EFSA
522 Journal 11:3145-3195
523 Ekinci FY, Baser GM, Özcan E et al (2016) Characterization of chemical, biological, and
524 antiproliferative properties of fermented black carrot juice, shalgam. European
525 Food Research and Technology 1-14
526 Ersus Bilek S, Yılmaz FM, Özkan G (2017) The effects of industrial production on black
527 carrot concentrate quality and encapsulation of anthocyanins in whey protein
528 hydrogels. Food and Bioproducts Processing 102:72-80
529 Ersus S, Yurdagel U (2007) Microencapsulation of anthocyanin pigments of black carrot
530 (Daucus carota L.) by spray drier. Journal of Food Engineering 80:805-812
531 Erten H, Tanguler H, Canbas A (2008) A traditional Turkish lactic acid fermented
532 beverage: Shalgam (salgam). Food Reviews International 24:352-359
533 Esatbeyoglu T, Rodriguez-Werner M, Schlösser A et al (2016) Fractionation of plant
534 bioactives from black carrots (Daucus carota subspecies sativus varietas
535 atrorubens Alef.) by adsorptive membrane chromatography and analysis of their

potential anti-diabetic activity. Journal of Agricultural and Food Chemistry 64:5901-5908

Fang Z, Bhandari B (2010) Encapsulation of polyphenols–a review. Trends in Food Science & Technology 21:510-523

Felgines C, Talavera S, Texier O et al (2006) Absorption and metabolism of red orange juice anthocyanins in rats. British Journal of Nutrition 95:898-904

Fernandes I, De Freitas V, Reis C et al (2012) A new approach on the gastric absorption of anthocyanins. Food & Function 3:508-516

Garcia-Herrera P, Pérez‐Rodríguez ML, Aguilera‐Delgado T et al (2016) Anthocyanin profile of red fruits and black carrot juices, purees and concentrates by HPLC‐DAD‐ESI/MS‐QTOF. International Journal of Food Science & Technology 51:2290-2300

Gertsch J (2009) How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. Journal of Ethnopharmacology 122:177-183

Gizir AM, Turker N, Artuvan E (2008) Pressurized acidified water extraction of black carrot [Daucus carota ssp. sativus var. atrorubens Alef.] anthocyanins. European Food Research and Technology 226:363-370

Gras CC, Bogner H, Carle R et al (2016) Effect of genuine non-anthocyanin phenolics and chlorogenic acid on color and stability of black carrot (Daucus carota ssp. sativus var. atrorubens Alef.) anthocyanins. Food Research International 85:291-300

Grassmann J, Schnitzler WH, Habegger R (2007) Evaluation of different coloured carrot cultivars on antioxidative capacity based on their carotenoid and phenolic contents. Int J Food Sci Nutr 58:603-611

561 Guldiken B, Boyacioglu D, Capanoglu E (2016) Optimization of extraction of bioactive
562 compounds from black carrot using response surface methodology (RSM). Food
563 Analytical Methods 9:1876–1886
564 Iliopoulou I, Thaeron D, Baker A et al (2015) Analysis of the thermal degradation of the
565 individual anthocyanin compounds of black carrot (Daucus carota L.): A new
566 approach using high-resolution proton nuclear magnetic resonance spectroscopy.
567 Journal of Agricultural and Food Chemistry 63:7066-7073
568 Kalt W (2005) Effects of production and processing factors on major fruit and vegetable
569 antioxidants. Journal of Food Science 70:R11-R19
570 Kamiloglu S, Capanoglu E, Bilen FD et al (2016a) Bioaccessibility of polyphenols from
571 plant-processing byproducts of black carrot (Daucus carota L.). Journal of
572 Agricultural and Food Chemistry 64:2450-2458
573 Kamiloglu S, Grootaert C, Capanoglu E et al (2017a) Anti-inflammatory potential of
574 black carrot (Daucus carota L.) polyphenols in a co-culture model of intestinal
575 Caco-2 and endothelial EA. hy926 cells. Molecular Nutrition & Food Research
576 61:
577 Kamiloglu S, Ozkan G, Isik H et al (2017b) Black carrot pomace as a source of
578 polyphenols for enhancing the nutritional value of cake: An in vitro digestion
579 study with a standardized static model. Lwt-Food Sci Technol 77:475-481
580 Kamiloglu S, Pasli AA, Ozcelik B et al (2015a) Colour retention, anthocyanin stability
581 and antioxidant capacity in black carrot (Daucus carota) jams and marmalades:
582 Effect of processing, storage conditions and in vitro gastrointestinal digestion.
583 Journal of Functional Foods 13:1-10

584 Kamiloglu S, Pasli AA, Ozcelik B et al (2015b) Influence of different processing and
585 storage conditions on in vitro bioaccessibility of polyphenols in black carrot jams
586 and marmalades. Food Chem 186:74-82
587 Kamiloglu S, Toydemir G, Boyacioglu D et al (2016b) A review on the effect of drying
588 on antioxidant potential of fruits and vegetables. Critical Reviews in Food Science
589 and Nutrition S110-S129
590 Kammerer D, Carle R, Schieber A (2003) Detection of peonidin and pelargonidin
591 glycosides in black carrots (Daucus carota ssp. sativus var. atrorubens Alef.) by
592 high-performance liquid chromatography/electrospray ionization mass
593 spectrometry. Rapid Communications in Mass Spectrometry 17:2407-2412
594 Kammerer D, Carle R, Schieber A (2004a) Characterization of phenolic acids in black
595 carrots (Daucus carota ssp. sativus var. atrorubens Alef.) by high-performance
596 liquid chromatography/electrospray ionization mass spectrometry. Rapid
597 Communications in Mass Spectrometry 18:1331-1340
598 Kammerer D, Carle R, Schieber A (2004b) Quantification of anthocyanins in black carrot
599 extracts (Daucus carota ssp. sativus var. atrorubens Alef.) and evaluation of their
600 color properties. European Food Research and Technology 219:479-486
601 Khandare V, Walia S, Singh M et al (2011) Black carrot (Daucus carota ssp. sativus)
602 juice: processing effects on antioxidant composition and color. Food and
603 Bioproducts Processing 89:482-486
604 Kirca A, Ozkan M, Cemeroglu B (2007) Effects of temperature, solid content and pH on
605 the stability of black carrot anthocyanins. Food Chem 101:212-218
606 Kocher G, Brar A, Dhillon T (2016) Fermentative production of alcoholic beverage from
607 black carrot. Agricultural Research Journal 53:138-140

608 Koley TK, Singh S, Khemariya P et al (2014) Evaluation of bioactive properties of Indian
609 carrot (Daucus carota L.): A chemometric approach. Food Research International
610 60:76-85
611 Kurilich AC, Clevidence BA, Britz SJ et al (2005) Plasma and urine responses are lower
612 for acylated vs nonacylated anthocyanins from raw and cooked purple carrots.
613 Journal of Agricultural and Food Chemistry 53:6537-6542
614 Lazcano CA, Yoo KS, Pike LM (2001) A method for measuring anthocyanins after
615 removing carotenes in purple colored carrots. Scientia Horticulturae 90:321-324
616 Lee EJ, Yoo KS, Patil BS (2011) Total carotenoid, anthocyanin, and sugar contents in
617 sliced or whole purple (cv. Betasweet) and orange carrots during 4-week cold
618 storage. Horticulture, Environment, and Biotechnology 52:402-407
619 Leja M, Kaminska I, Kramer M et al (2013) The content of phenolic compounds and
620 radical scavenging activity varies with carrot origin and root color. Plant Foods
621 for Human Nutrition 68:163-170
622 Metzger BT, Barnes DM, Reed JD (2008) Purple carrot (Daucus carota L.)
623 polyacetylenes decrease lipopolysaccharide-induced expression of inflammatory
624 proteins in macrophage and endothelial cells. Journal of Agricultural and Food
625 Chemistry 56:3554-3560
626 Minihane AM, Vinoy S, Russell WR et al (2015) Low-grade inflammation, diet
627 composition and health: current research evidence and its translation. British
628 Journal of Nutrition 114:999-1012
629 Montilla EC, Arzaba MR, Hillebrand S et al (2011) Anthocyanin composition of black
630 carrot (Daucus carota ssp. sativus var. atrorubens Alef.) cultivars antonina, beta
631 sweet, deep purple, and purple haze. Journal of Agricultural and Food Chemistry
632 59:3385-3390

633 Murali S, Kar A, Mohapatra D et al (2015) Encapsulation of black carrot juice using spray
634 and freeze drying. Food Science and Technology International 21:604-612
635 Nayak B, Liu RH, Tang J (2015) Effect of processing on phenolic antioxidants of fruits,
636 vegetables, and grains—A review. Critical Reviews in Food Science and
637 Nutrition 55:887-918
638 Netzel M, Netzel G, Kammerer DR et al (2007) Cancer cell antiproliferation activity and
639 metabolism of black carrot anthocyanins. Innovative Food Science & Emerging
640 Technologies 8:365-372
641 Nicoli M, Anese M, Parpinel M (1999) Influence of processing on the antioxidant
642 properties of fruit and vegetables. Trends in Food Science & Technology 10:94-
643 100
644 Novotny JA, Clevidence BA, Kurilich AC (2012) Anthocyanin kinetics are dependent on
645 anthocyanin structure. British Journal of Nutrition 107:504-509
646 Olejnik A, Rychlik J, Kidon M et al (2016) Antioxidant effects of gastrointestinal
647 digested purple carrot extract on the human cells of colonic mucosa. Food Chem
648 190:1069-1077
649 Ozen G, Akbulut M, Artik N (2011) Stability of black carrot anthocyanins in the Turkish
650 delight (lokum) during storage. Journal of Food Process Engineering 34:1282-
651 1297
652 Padayachee A, Netzel G, Netzel M et al (2013) Lack of release of bound anthocyanins
653 and phenolic acids from carrot plant cell walls and model composites during
654 simulated gastric and small intestinal digestion. Food & Function 4:906-916
655 Park S, Kang S, Jeong DY et al (2015) Cyanidin and malvidin in aqueous extracts of
656 black carrots fermented with Aspergillus oryzae prevent the impairment of

energy, lipid and glucose metabolism in estrogen-deficient rats by AMPK activation. Genes & Nutrition 10:1-14

Park S, Kang S, Jeong DY et al (2016) Black carrots fermented with Lactobacillus plantarum or Aspergillus oryzae prevent cognitive dysfunction by improving hippocampal insulin signalling in amyloid-β infused rats. Journal of Functional Foods 25:354-366

Passamonti S, Vrhovsek U, Vanzo A et al (2003) The stomach as a site for anthocyanins absorption from food. FEBS Letters 544:210-213

Patras A, Brunton NP, O'Donnell C et al (2010) Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends in Food Science & Technology 21:3-11

Poudyal H, Panchal S, Brown L (2010) Comparison of purple carrot juice and β-carotene in a high-carbohydrate, high-fat diet-fed rat model of the metabolic syndrome. British Journal of Nutrition 104:1322-1332

Reyes LF, Cisneros-Zevallos L (2007) Degradation kinetics and colour of anthocyanins in aqueous extracts of purple-and red-flesh potatoes (Solanum tuberosum L.). Food Chem 100:885-894

Rothwell JA, Medina-Remon A, Perez-Jimenez J et al (2015) Effects of food processing on polyphenol contents: A systematic analysis using Phenol ‐ Explorer data. Molecular Nutrition & Food Research 59:160-170

Sadilova E, Carle R, Stintzing FC (2007) Thermal degradation of anthocyanins and its impact on color and in vitro antioxidant capacity. Molecular Nutrition & Food Research 51:1461-1471

Sadilova E, Stintzing FC, Kammerer DR et al (2009) Matrix dependent impact of sugar and ascorbic acid addition on color and anthocyanin stability of black carrot,

682 elderberry and strawberry single strength and from concentrate juices upon
683 thermal treatment. Food Research International 42:1023-1033
684 Schwarz M, Wray V, Winterhalter P (2004) Isolation and identification of novel
685 pyranoanthocyanins from black carrot (Daucus carota L.) juice. Journal of
686 Agricultural and Food Chemistry 52:5095-5101
687 Singh PP, Saldana MD (2011) Subcritical water extraction of phenolic compounds from
688 potato peel. Food Research International 44:2452-2458
689 Siro I, Kapolna E, Kapolna B et al (2008) Functional food. Product development,
690 marketing and consumer acceptance—A review. Appetite 51:456-467
691 Sun T, Simon PW, Tanumihardjo SA (2009) Antioxidant phytochemicals and antioxidant
692 capacity of biofortified carrots (Daucus carota L.) of various colors. Journal of
693 Agricultural and Food Chemistry 57:4142-4147
694 Suzme S, Boyacioglu D, Toydemir G et al (2014) Effect of industrial juice concentrate
695 processing on phenolic profile and antioxidant capacity of black carrots.
696 International Journal of Food Science & Technology 49:819-829
697 Talavera S, Felgines C, Texier O et al (2003) Anthocyanins are efficiently absorbed from
698 the stomach in anesthetized rats. The Journal of Nutrition 133:4178-4182
699 Turker N, Aksay S, Ekiz HI (2004) Effect of storage temperature on the stability of
700 anthocyanins of a fermented black carrot (Daucus carota var. L.) beverage:
701 shalgam. Journal of Agricultural and Food Chemistry 52:3807-3813
702 Turker N, Erdogdu F (2006) Effects of pH and temperature of extraction medium on
703 effective diffusion coefficient of anthocynanin pigments of black carrot (Daucus
704 carota var. L.). Journal of Food Engineering 76:579-583

705 Turkyilmaz M, Ozkan M (2012) Kinetics of anthocyanin degradation and polymeric
706 colour formation in black carrot juice concentrates during storage. International
707 Journal of Food Science & Technology 47:2273-2281
708 Turkyilmaz M, Yemis O, Ozkan M (2012) Clarification and pasteurisation effects on
709 monomeric anthocyanins and percent polymeric colour of black carrot (Daucus
710 carota L.) juice. Food Chem 134:1052-1058
711 Wallace TC, Giusti MM (2008) Determination of color, pigment, and phenolic stability
712 in yogurt systems colored with nonacylated anthocyanins from Berberis boliviana
713 L. as compared to other natural/synthetic colorants. Journal of Food Science
714 73:C241-C248
715 Witrowa-Rajchert D, Bawol A, Czapski J et al (2009) Studies on drying of purple carrot
716 roots. Drying Technology 27:1325-1331
717 Wright OR, Netzel GA, Sakzewski AR (2013) A randomized, double-blind, placebo-
718 controlled trial of the effect of dried purple carrot on body mass, lipids, blood
719 pressure, body composition, and inflammatory markers in overweight and obese
720 adults: The QUENCH Trial 1. Canadian Journal of Physiology and Pharmacology
721 91:480-488
722 Zozio S, Pallet D, Dornier M (2011) Evaluation of anthocyanin stability during storage
723 of a coloured drink made from extracts of the Andean blackberry (Rubus glaucus
724 Benth.), açai (Euterpe oleracea Mart.) and black carrot (Daucus carota L.). Fruits
725 66:203-215

726 **Figure legends**

727 **Figure 1.** Chemical structures of the anthocyanins detected in the black carrots: (1)
728 cyanidin 3-xylosyl-glucosyl-galactoside, (2) cyanidin 3-xylosyl-galactoside, (3) sinapic
729 acid derivative of cyanidin 3-xylosylglucosylgalactoside, (4) ferulic acid derivative of
730 cyanidin 3-xylosylglucosylgalactoside, (5) coumaric acid derivative of cyanidin 3-
731 xylosyl-glucosyl-galactoside, (6) peonidin 3-xylosyl-glucosyl-galactoside, (7) ferulic
732 acid derivative of pelargonidin 3-xylosyl-glucosyl-galactoside, and (8) ferulic acid
733 derivative of peonidin 3-xylosyl-glucosyl-galactoside (Algarra et al. 2014).

734 **Figure 2.** Chemical structure of chlorogenic acid (5-*O*-caffeoylquinic acid).

Table 1. Nutrient content of black carrots (per 100 g edible food) (TurKomp, http://www.turkomp.gov.tr)

Component	Unit	Mean	Minimum	Maximum
Energy	kcal	42	41	43
Energy	kJ	175	171	179
Water	g	87.66	87.29	88.02
Ash	g	0.84	0.76	0.92
Protein	g	0.87	0.75	1.00
Nitrogen	g	0.14	0.12	0.16
Fat, total	g	0.14	0.12	0.16
Carbohydrate	g	8.01	8.00	8.02
Fiber, total dietary	g	2.48	2.35	2.61
Fiber, water-soluble	g	0.90	0.84	0.97
Fiber, water-insoluble	g	1.58	1.51	1.65
Saccharose	g	0.00	0.00	0.00
Glucose	g	1.85	1.85	1.85
Fructose	g	0.14	0.14	0.14
Lactose	g	0.00	0.00	0.00
Maltose	g	0.00	0.00	0.00
Salt	mg	206	196	215
Iron, Fe	mg	0.26	0.24	0.28
Phosphorus, P	mg	29	21	38
Calcium, Ca	mg	33	31	35
Magnesium, Mg	mg	17	17	18
Potassium, K	mg	256	240	273
Sodium, Na	mg	82	78	86
Zinc, Zn	mg	0.15	0.14	0.17
Thiamin	mg	0.029	0.026	0.032
Riboflavin	mg	0.029	0.022	0.035
Niacin, preformed	mg	1.211	1.114	1.308
Vitamin B6, total	mg	0.072	0.064	0.079
Vitamin A	RE	0	0	0
Beta-carotene	µg	0	0	0
Lycopene	µg	0	0	0
Lutein	µg	0	0	0

735

Table 2. Studies determining the cytotoxicity of black carrot polyphenols on various cell lines

Sample	Assay	Cell type	Treatment period	Dose-response	Reference
Black carrot anthocyanin-rich extract	MTT	HL-60	24 h	0.5 mg/mL -> 63% 1 mg/mL -> 49% 1.5 mg/mL -> 28% 2 mg/mL -> 23%	Netzel et al. 2007
		HT-29	24 h	0.5 mg/mL -> 91% 1 mg/mL -> 65% 1.5 mg/mL -> 42% 2 mg/mL -> 25%	
Black carrot and shalgam extracts	MTS	Caco-2	48 h	0.05 mg black carrot/mL -> 96.7% 0.05 mg shalgam/mL -> 97.7% 6.4 mg black carrot/mL -> 62.1% 6.4 mg shalgam/mL -> 59.3%	Ekinci et al. 2016
Deep purple carrot extract	LDH	Caco-2/TC7	24 h	0.1 mg extract was non-toxic.	Esatbeyoglu et al. 2016
Non-digested and gastrointestinal digested purple carrot extracts	MTT	NCM460	48 h	4.84 mg non-digested/mL -> 90% 3.73 mg digested/mL -> 90% 14.49 mg non-digested/mL -> 50% 10.09 mg digested/mL -> 50%	Olejnik et al. 2016
Undigested and digested black carrot extracts	MTT	Caco-2	4 h	5 mg non-digested/mL -> 93.4% 0.1 mg digested/mL -> 99.9%	Kamiloglu et al. 2017

Caco-2: human colorectal adenocarcinoma cell line; HL-60: human promyelocytic leukemia cells; HT-29: colorectal adenocarcinoma cells; LDH: lactate dehydrogenase; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NCM460: human colon mucosal epithelial cell line; RAW264.7: murine macrophage cell line.

Table 3. Studies investigating the effect of processing on black carrot polyphenols

Sample	Processing conditions	Major conclusions	Reference
Black carrot extract	Microencapsulation by spray drying (160–200 °C)	• Higher air inlet temperatures caused higher ACN losses. • 20–21 DE maltodextrin gave the highest ACN content.	(Ersus and Yurdagel 2007)
Black carrot concentrate	Thermal treatment at 70–90 °C, 11–64 °Brix and pH 4.3, 6.0	• ACN degradation followed a first-order reaction model. • The degradation rate of ACNs increased with increased heating temperature and solid content.	(Kirca et al. 2007)
Purified black carrot anthocyanins	Heating at 95°C and pH 3.5 for 6 h	• Acylated ACNs were more resistant to degradation compared to non-acylated ACNs. • Acylated ACNs are cleaved into the corresponding acyl-glycosides and the aglycones the latter being readily transformed into the chalcone, which is cleaved into protocatechuic acid and phloroglucinaldehyde.	(Sadilova et al. 2007)
Black carrot juice	Sugar and ascorbic acid addition	• The retention of black carrot ACNs upon saccharide and ascorbic acid supplementation ranged from 50.4% to 58.1%, with the retention of unsupplemented juices being 48.3%.	(Sadilova et al. 2009)
Purple carrot roots	Convective drying (70 °C, 1.5 m/s), microwave-convective drying (40 °C, 300 W), infrared convective drying (7.875 kW/m^2, 1.2 m/s), and freeze drying (–70 °C, 63 Pa)	• For Deep Purple cultivar, the most advantageous drying method was microwave-convection drying (20% reduction in ACNs), whereas convection drying caused the highest degradation (about 50%). • For Purple Haze cultivar, the most advantageous method was freeze-drying (30% loss of ACNs), while infrared convective drying was the least advantageous.	(Witrowa-Rajchert et al. 2009)
Black carrot juice	Pre-press maceration treatment with pectinase	• Enzyme-assisted processing increased the TPC (27%), TFC (46%) and ACNs (100%).	(Khandare et al. 2011)

(Continues)

Table 3. Continues

Sample	Processing conditions	Major conclusions	Reference
Black carrot juice	Clarification and pasteurization	• Depectinization and bentonite treatments resulted in increases (7% and 20%, respectively) in ACNs. • Gelatine–kieselsol treatment and pasteurization resulted in reduction (10% and 3–16%) in ACNs.	(Turkyilmaz et al. 2012)
Black carrot	Concentrate processing	• Processing black carrot into concentrate led to an overall reduction of 70%, 73% and 44% in TPC, TFC and ACNs, respectively.	(Suzme et al. 2014)
Black carrot juice	Clarification and pasteurization	• Depectinization (13 and 59%) and pasteurization (1.1- and 2.3-fold) led to increases in the TPC and HCAs. • Bentonite (10 and 7%) and gelatin–kieselsol (25 and 29%) treatments led to reductions in TPC and HCAs.	(Dereli et al. 2015)
Black carrot powder and solution	Freeze drying at pH 3.5 and 6.8 and thermal treatment at 180 ℃ for 1 h	• At pH 6.8, there was only a small overall increase in ACN degradation compared to pH 3.6. • For the samples at pH 3.6, the degradation properties of ACNs in the powder were similar to those of the solution, whereas at pH 6.8 powder was significantly more stable than in solution. • At pH 6.8, the stability of the acylated ACNs was markedly higher in the powder than in the solution.	(Iliopoulou et al. 2015)
Black carrot	Jam and marmalade (55 and 68°Brix, respectively) processing with sugar or sweeneter	• Jam and marmalade processing significantly decreased TPC (89.2–90.5%), ACNs (87.6–95.6%) and PAs (49.5–96.7%). • Use of sweetener instead of sugar in jams and marmalades did not lead to a statistically significant difference in polyphenol content.	(Kamiloglu et al. 2015a; b)
Black carrot juice	Encapsulation with spray drying (150–225 ℃) and freeze drying (–53 °C, 0.11–0.22 mbar)	• Maltodextrin 20DE as the carrier material has proven to be better in retaining ACNs compared to gum arabic and tapioca starch. • The best spray dried product, was obtained at 150 ℃. • Freeze dried product was the most acceptable one with maximum ACNs.	(Murali et al. 2015)

(Continues)

Table 3. Continues

Sample	Processing conditions	Major conclusions	Reference
Black carrot juice	Thermal treatment at 90 ℃ for 5 h	• ACN stability during heating was barely improved unless the concentrations of co-pigments exceeded those of their natural source.	(Gras et al. 2016)
Black carrot	Fermentative alcoholic beverage production	• There was a significant increase in TPC (29%), while the increase in ACNs was not significant (<1%).	(Kocher et al. 2016)
Black carrot	Concentrate processing and microencapsulation in whey protein hydrogels	• The degradation rates of TPC, TFC, and ACNs of black carrots from raw material to concentrate were 25%, 20% and 19%, respectively. • ACNs were entrapped more than other phenolic compounds.	(Ersus Bilek et al. 2017)

ACN: anthocyanin; HCA: hydroxycinnamic acid; PA: phenolic acids; TFC: total flavonoid content; TPC: total phenolic content.

Table 4. Studies investigating the effect of storage on black carrot polyphenols

Sample	Storage conditions	Major conclusions	Reference
Fermented black carrot beverage (Shalgam)	4, 25, and 40 °C for 90 days	• The effect of 40 °C storage temperature ACNs was significant, while there was no significant difference between 4 and 25 °C. • Acylated ACNs retained 7.9–48.9% of their initial level, whereas nonacylated ACNs retained only 0–11.1% of their initial level after storage at 40 °C. • There were no significant effects of pasteurization and sorbate treatment on the total ACN content or the ACN profile with respect to control at any storage temperature.	(Turker et al. 2004)
Ready-to-eat shredded purple carrots	Chilled temperature (5 ± 2 °C) in air, or in modified atmosphere packaging (90%N_2+5%O_2+5%CO_2 and 95%O_2+5%CO_2) for 13 days	• ACNs showed no significant decrease for all treatments over the entire storage period, except for modified atmosphere packaging treatment (95%O_2 +5%CO_2), which significantly decreased on day 13 (≈10%).	(Alasalvar et al. 2005)
Microencapsulated pigments of black carrot	4 and 25 °C	• Storage at 4 °C increased half life of spray dried ACN pigments 3 times more compared to 25 °C storage temperature.	(Ersus and Yurdagel 2007)
Black carrot concentrate	4, 20 and 37 °C and 30, 45 and 64 °Brix	• ACN degradation during storage fitted a to first-order reaction model. • Storage at 37 °C resulted in a much faster ACN degradation as compared to storage at 4 °C ($t_{1/2}$ = 4.0–4.5 and 71.8–215 weeks, respectively).	(Kirca et al. 2007)
Yoghurt with purple carrot extract	4 °C for 8 weeks	• A significant decrease in ACNs was noted in all yogurt fat matrices over the storage period ($p < 0.01$). • ACN degradation followed first order kinetics. • Yogurt matrices containing higher fat contents showed increased stability of ACNs.	(Wallace and Giusti 2008)

(Continues)

Table 4. Continues

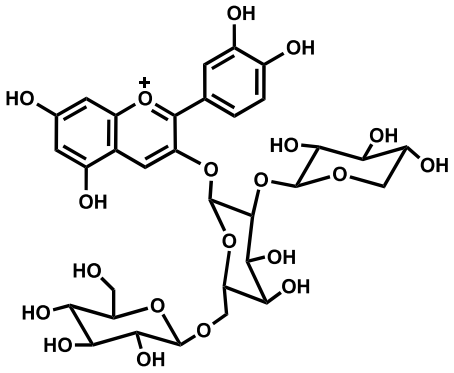
Sample	Storage conditions	Major conclusions	Reference
Sliced purple carrots	2, 4 °C for 4 weeks	• ACNs did not change significantly at either temperature.	(Lee et al. 2011)
Turkish delight colored with black carrot juice concentrate	12, 20 and 30 °C for 5 months	• Kinetic data suggested a first-order reaction for the degradation of ACNs. • Degradation rates of ACNs increased with increasing temperature.	(Ozen et al. 2011)
Soft drink colored with black carrot extract	4, 20, 30 and 50 °C for 60 days	• No degradation was detected during the refrigerated storage (4 °C). • Degradation of the ACNs at higher temperatures followed a first-order kinetic model. • Black carrot ACNs degraded more slowly than açai and blackberry ACNs during thermal storage.	(Zozio et al. 2011)
Black carrot juice concentrate	−23, 5 and 20 °C for 319 days and at 30 °C for 53 days	• ACN degradation was fitted to a first-order reaction model. • Acylated ACNs were more stable during storage. • At sub-freezing temperatures ACN degradation was minimum.	(Turkyilmaz and Ozkan 2012)
Black carrot jams and marmalades	4 and 25 °C for 20 weeks	• The reduction in TPC and ACNs in samples stored at 25°C (26.4–48.0% and 30.7–92.2%, respectively) was higher than samples stored at 4°C (21.0–42.5% and 19.0–46.4%, respectively). • Acylated ACNs were significantly more stable than non-acylated ACNs.	(Kamiloglu et al. 2015a; b)

ACN: anthocyanin; $t_{1/2}$: time needed for 50% degradation of anthocyanins; TPC: total phenolic content.

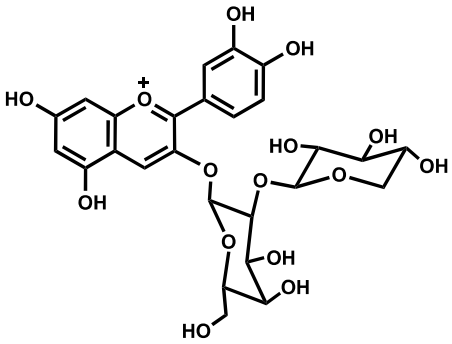
Table 5. Human studies on bioavailability of black carrot polyphenols

Treatment	Dose	Design	Major conclusions	Reference
Raw and cooked purple carrot	250 g raw carrot (463 μmol ACNs); 250 and 500 g cooked carrot (357 and 714 μmol ACNs)	Blood sampling for 8 h Urine sampling for 24 h	• Four of the five carrot ACNs were found intact in plasma and urine. • Acylation of ACNs resulted in 11-14-fold and 8-10-fold decreases in ACN recovery in urine and plasma, respectively. • Cooking increased the recovery of non-acylated ACNs, but not acylated ACNs. • Increased dose reduced the recovery of both acylated and nonacylated ACNs.	(Kurilich et al. 2005)
Black carrot concentrate	100 mL concentrate (672.5 mg ACNs)	Urine sampling for 24 h	• Three acylated and two nonacylated ACNs were excreted in urine. • Cyanidin-monoglucuronide and and cyanidin-monosulfate were identified as metabolites. • Urine recovery of nonacylated ACNs was 8-fold higher than that of acylated ACNs.	(Netzel et al. 2007)
Purple carrot juice	50, 150, and 250 mL juice (76, 228, and 380 μmol ACNs)	Plasma sampling for 8 h	• Plasma concentrations of nonacylated ACNs were 4-fold higher than that for acylated ACNs. • Absorption efficiency declined across the doses administered.	(Charron et al. 2009)
Raw and cooked purple carrot	250 g raw carrot (463 μmol ACNs); 250 and 500 g cooked carrot (357 and 714 μmol ACNs)	Blood sampling for 8 h Urine sampling for 24 h	• Four of the five carrot ACNs were found intact in plasma and urine. • Absorption efficiencies of acylated ACNs were less than those for non-acylated ACNs. • The acylated ACNs exhibited a shorter half-life for gastrointestinal absorption than the non-acylated ACNs.	(Novotny et al. 2012)

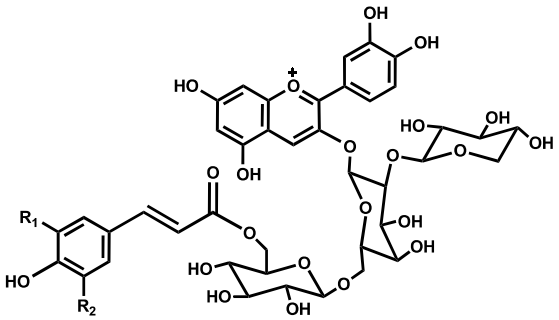
ACN: anthocyanin



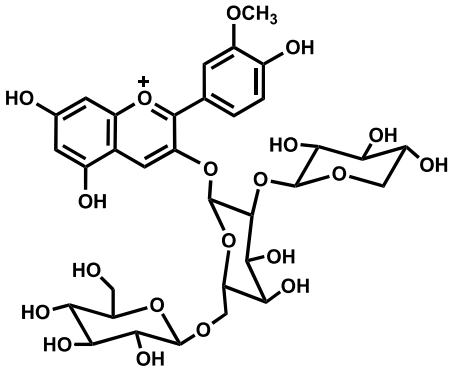
1) cyanidin 3-xylosylglucosylgalactoside



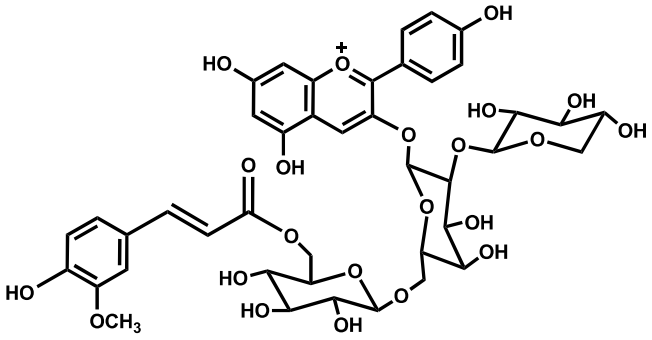
2) cyanidin 3-xylosylgalactoside



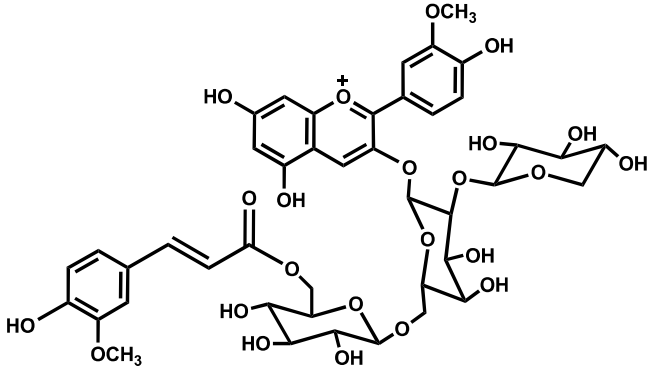
3) R_1=R_2=OCH_3: sinapic acid derivative of cyanidin 3-xylosylglucosylgalactoside
4) R_1=H; R_2=OCH_3: ferulic acid derivative of cyanidin 3-xylosylglucosylgalactoside
5) R_1=R_2=H: coumaric acid derivative of cyanidin 3-xylosylglucosylgalactoside



6) peonidin 3-xylosylglucosylgalactoside



7) ferulic acid derivative of pelargonidin 3-xylosylglucosylgalactoside



8) ferulic acid derivative of peonidin 3-xylosylglucosylgalactoside

Figure 1

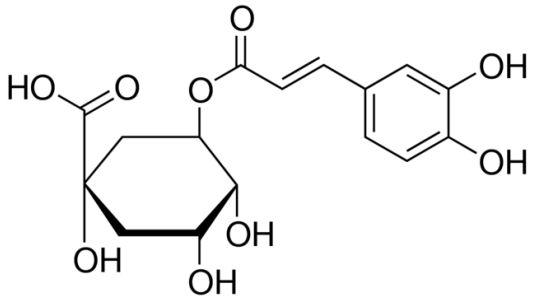


Figure 2