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Carotenoids, polyphenols and micronutrient profiles of *Brassica oleraceae* and plum varieties and their contribution to measures of total antioxidant capacity

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ABSTRACT

The consumption of phytochemicals such as carotenoids and polyphenols within whole fruits and vegetables has been associated with decreased incidence of various inflammation and oxidative stress related chronic diseases, which may be due to direct antioxidant effects, or indirect mechanisms such as affecting signal transduction/gene expression. Within the present study, we investigated the antioxidant composition of two major groups of vegetables and fruits, *Brassica oleraceae* and *prunus* spp., and estimated their contribution to antioxidant capacity. For this purpose, 17 plum and 27 *Brassica* varieties were collected in Luxembourg, and analysed for their individual polyphenol and carotenoid profile, vitamin C, dietary fibre, and minerals/trace elements, and their correlation with markers of antioxidant capacity (FRAP, ABTS, Folin–Ciocalteu). Total carotenoid and polyphenol content varied considerably between the different *Brassica* and plum varieties, with highest concentrations in the variety Kale (13.3 ± 0.58 mg/100 g wet weight) and Cherry plum (1.96 ± 0.28 mg/100 g) for carotenoids; and Kale (27.0 ± 0.91 mg/100 g) and Kirks plum (185 ± 14 mg/100 g) for polyphenols. In developed multiple linear-regression-models for *Brassica*, flavonoids, anthocyanins, lutein and vitamin C were found to be the best predictors of antioxidant capacity as assessed by FRAP (R^2 = 0.832) and flavonoids, neochlorogenic acid and vitamin C as assessed by ABTS (R^2 = 0.831); while for plums these were selenium, total sugars, chlorogenic acid and vitamin C (R^2 = 0.853), and selenium, chlorogenic acid and flavonoids for FRAP (R^2 = 0.711). When considering *Brassica* and plum consumption in Luxembourg, it is estimated that both contribute to an antioxidant intake equivalent to 26 and 6 mg per day of ascorbic acid equivalents, respectively.

1. Introduction

It is generally recommended by health organizations such as the WHO that fruits and vegetables should be frequently incorporated into the diet as part of a healthy lifestyle. Several epidemiological studies have shown that the long term intake of fruits and vegetables is significantly correlated with a decreased risk of developing inflammation and oxidative stress related chronic diseases, including cardiovascular diseases (D'Odorico et al., 2000), type 2 diabetes (Bazzano, Li, Joshipura, & Hu, 2008) and cancer (Eliassen et al., 2012). Fruits and vegetables, also in form of processed products (i.e. tea, coffee, chocolate, and red wine) contain significant amounts of various phytochemicals, which have been postulated to provide health beneficial effects, reducing the risk of chronic diseases. There exist far over >10,000 different phytochemicals such as carotenoids, polyphenols, and phytosterols. However until today, it is poorly comprehended which of these are the most bioactive with respect to the attributed health effects.

Carotenoids and polyphenols constitute 2 predominant classes of phytochemicals, both possessing properties that have been re-

Abbreviations: AAPH, 2-2′-azobis (2-methylpropionamidine) dichloride; ABTS, 2-2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; $AlCl_3$, aluminium chloride; CE, catechin equivalent; CGE, cyanidin 3-glucoside equivalents; CTAB, cetyl trimethylammonium bromide; DI, 2,6 dichlorophenolindophenol; FRAP, Ferric-reducing antioxidant power assay; GAE, gallic acid equivalents; HCl, hydrogen chloride; HNO_3, nitric acid; H_2SO_4, sulphuric acid; Na_2CO_3, sodium carbonate; $NaNO_2$, sodium nitrite; NaOH, sodiumhydroxide; MTBE, tert-butyl methyl ether; PBS, phosphate buffer saline; VCEAC, vitamin C equivalent antioxidant capacity; VCE, vitamin C equivalents; WHO, World Health Organization.

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lated to health benefits, such as via impacting cellular signalling cascades or their antioxidant properties (Palozza, Serini, Ameruso, & Verdecchia, 2012). Carotenoids are, together with terpenes, the most predominant fat-soluble secondary plant metabolites. Over 700 carotenoids have been identified, but only around 100 play a role in the daily diet (Bohn, 2008). Polyphenols represent the largest group of water-soluble phytochemicals. As exogenous antioxidants, both take part in antioxidant defense mechanisms, preventing damaging effects of reactive oxygen species (ROS) on DNA, proteins, and lipids. While carotenoids are involved in the scavenging of singlet molecular oxygen ($^1O_2$) and peroxyl radicals, especially in the lipid bilayer, the antioxidant capacity of polyphenols depends on their ability to scavenge either free radicals or lipid peroxyl radicals as well as by acting as singlet oxygen quenchers (Bouayed & Bohn, 2010), predominantly in aqueous compartments such as the cytosol. More recently, several studies have demonstrated that polyphenols and carotenoids significantly down-regulated the expression of pro-inflammatory cytokines, possibly due to alterations of the NF-κB pathway (Kim, Seo, & Kim, 2011; Romier, Van de Walle, During, Larondelle, & Schneider, 2008; Vazquez-Agell et al., 2013), and impacted Nrf2, a transcription factor related to the expression of detoxifying agents such as phase II enzymes, meaning that they might act, in addition to directly quenching ROS, as indirect antioxidants, which may in fact be their primary activity *in vivo*.

Carotenoid and polyphenol intake and serum concentrations have been inversely associated with reduced incidence of chronic diseases such as type 2 diabetes (Coyne et al., 2005). Due to negative results following intervention studies with isolated compounds (de Maat, Pijl, Kluft, & Princen, 2000; Hininger et al., 2001), it has been argued that the effects rest rather in the entire plethora of phytochemicals and/or micronutrients present in fruits or vegetables, rather than the consumption of an individual phytochemical.

Carotenoids are found at high concentrations in a variety of coloured vegetables and some fruits, ranging from 0.1 to >2 mg/100 g. In a normal diet, humans consume approx. 4–15 mg carotenoids per day and capita (Biehler et al., 2012). By contrast, the daily intake of polyphenols ranges 2–3 magnitudes higher, up to 1 g per day per capita (Scalbert & Williamson, 2000). Nevertheless, due to their low absorption and high metabolism, plasma carotenoid concentrations are several μmol/L (Bohn, 2008), while those of polyphenols may be lower (Scalbert & Williamson, 2000). However, the production of local fruits and vegetables plays an integral part for supplying healthy foods rich in micronutrients and phytochemicals. Luxembourg has been putting much emphasis on the production of regional products, but only 1% of fruits and vegetables consumed in Luxembourg are also produced there (Ministère de l'Agriculture, 2009). In 2004, 8.328 tons of fruits (Le portail des Statistiques, 2012b) and 2.750 tons of vegetables (Le portail des Statistiques, 2012a) have been commercialized in Luxembourg, with *Prunus* species (including plums and mirabelles) representing 7% of the produced fruits, second only to apples, with a consumption (as fresh fruits) of 16 kg/year. For vegetables, *Brassica oleraceae* species such as broccoli, cabbage, turnip and cauliflower represent ca. 3% of the produced vegetables, ranking eighth among the consumed vegetables (13.8 kg/year).

Both *Brassica oleraceae* and plum can contain relatively high concentrations of carotenoids and polyphenols, e.g. ca. 2 mg/100 g (Biehler et al., 2012) and 30–60 mg/100 g, respectively, in kale (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004), and 0.4 mg/100 g and 115 mg/100 g, respectively, for plums (Manach et al., 2004; Souci, Fachmann, & Kraut, 2000). In the present study, we estimated the contribution of present phytochemicals (carotenoids and polyphenols), selected micro- and macronutrients to antioxidant activity and further estimated the contribution of *Brassica oleraceae* and plum consumption to the intake of antioxidants.

2. Materials and methods

2.1. Chemicals and standards

All products were of analytical grade or higher. 18 MΩ water was prepared with a purification system from Millipore (Brussels, Belgium) and was used throughout. Unless otherwise stated, all chemicals including aluminium chloride ($AlCl_3$), 2-2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2-2′-azobis (2-methylpropionamidine) dichloride (AAPH), (+)-catechin, gallic acid, Folin–Ciocalteu's phenol reagent, alpha-amylase, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), ascorbic acid (vitamin C) and 2,6 dichlorophenolindophenol were procured from Sigma–Aldrich (St. Louis, MO, USA). Iron(III)chloride 6-hydrate, sodium hydroxide (NaOH), sodium acetate, acetone, cetyl trimethylammonium bromide (CTAB), sodium carbonate (Na_2CO_3) and potassium chloride were obtained from Merck (Darmstadt, Germany). Acetic acid, hydrogen chloride (HCl), ammonium acetate, sulphuric acid (H_2SO_4), sodium nitrite ($NaNO_2$) and oxalic acid were from VWR (Leuven, Belgium). Methanol was procured from Biosolve (Valkenswaard, The Netherlands). Nitric acid (HNO_3) was procured from SCP Science (Courtaboeuf, France).

Carotenoid standards of β-carotene, β-cryptoxanthin and lutein were purchased from Extrasynthèse (Lyon, France), α-carotene, violaxanthin, neoxanthin, phytoene and phytofluene were purchased from CaroteNature (Lupsingen, Switzerland), trans-β-apocarotenal was from Sigma–Aldrich. Certified purity of all standard was above 95%. Polyphenol standards, i.e. (+)-catechin, caffeic acid, p-coumaric acid, cryptochlorogenic acid (4-caffeoylquinic acid), neochlorogenic acid (3-caffeoylquinic acid), quercetin, quercetin 3-*O*-galactoside, kaempferol, kaempferol 3-*O*-glucoside, ferulic acid, syringic acid, sinapic acid, gallic acid and vanillic acid were obtained from Sigma–Aldrich. Chlorogenic acid and cinnamic acid were purchased from Merck. Kaempferol-3-*O*-rutinoside was obtained from Extrasynthèse and 4-hydroxybenzoic acid was purchased from Thermo Fisher Scientific (Geel, Belgium).

2.2. Sample preparation

Twenty-seven *Brassica oleraceae* varieties and 17 plum varieties were procured from different local Luxembourgish farmers or markets (Table 1) between September 2010 and November 2011. *Brassica* varieties (n = 1 piece per variety except for Brussels sprouts (n = 4–6), and cauliflower and broccoli (n = 2–3) were purchased fresh from markets, and stored for analysis for a maximum of 2 weeks prior to further processing. Plums were likewise obtained in fresh form from various markets, and aliquots of 6–9 plums per variety were chosen for further processing, with storage at a maximum of 1 week at 4 °C prior to processing.

For each *Brassica* variety, all edible leaves were carefully separated, packed into transparent polyethylene bags, dipped in liquid nitrogen and stored at −80 °C. For each plum variety, the plums were cut into half with a sharp knife, the kernel removed, packed into transparent polyethylene bags, dipped in liquid nitrogen and stored at −80 °C. Samples were then further lyophilised (Christ freeze dryer, Thermo Fisher Scientific, Geel, Belgium) during 24 h, combined and homogenized with a grinder (Mortar Grinder RM 200, Retsch, Aartselaar, Belgium) and stored in 50-mL plastic centrifuge tubes at −20 °C until analysis.

2.3. Extraction of phenolic compounds

For the extraction of phenolics a protocol similar as described earlier was followed (Bouayed, Hoffmann, & Bohn, 2011). Three independent extractions for each variety were carried out on ice