Food Chemistry 203 (2016) 23–27

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Short communication

In vitro liberation of carotenoids from spinach and Asia salads after different domestic kitchen procedures

Jane N. Eriksen [a,b], Amy Y. Luu [a], Lars O. Dragsted [a], Eva Arrigoni [b,*]

[a] University of Copenhagen, Department of Nutrition, Exercise and Sports, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark
[b] Agroscope, Institute of Food Sciences, Schloss 1, CH-8820 Wädenswil, Switzerland

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 1 February 2016
Accepted 3 February 2016
Available online 4 February 2016

Keywords:
Lutein
β-Carotene
Spinach
Asia salads
Domestic heat treatments
Release from matrix

ABSTRACT

Green-leafy vegetables are rich in nutritionally important constituents including carotenoids. Their potential health benefits depend among others on their liberation from the plant matrix. The aim of the present study was to evaluate the effect of particle size and heat treatments on lutein and β-carotene liberation from spinach and Asia salads by applying an *in vitro* digestion protocol and UHPLC analysis. Reduction of particle size resulted in a three- to fourfold increase in liberation of lutein and β-carotene when comparing whole leaf and puree preparations of spinach. However, this positive effect was shown to be nullified by the severe heat impact during stir-frying of minced spinach, showing that domestic treatments need to be chosen carefully to maximise carotenoid liberation. Steaming significantly improved lutein liberation from Asia salads, but had no or a negative effect in spinach samples, possibly due to differences in liberation or degradation between the two plant matrices.

1. Introduction

Carotenoids are of importance as naturally occurring yellow to orange fat-soluble pigments (Maiani et al., 2009; Rao & Rao, 2007). As plant food components, they are associated with a decrease in the risk of chronic disorders such as cardiovascular diseases (Granado, Olmedilla, & Blanco, 2003) and specific types of cancer (Finley, 2005). Moreover, xanthophylls have a potential role in prevention and treatment of certain eye diseases such as age-related macular degeneration (AMD) and cataracts (Ma & Lin, 2010).

Dark green leafy vegetables are high in lutein and β-carotene and spinach consumption has been reported to increase both plasma lutein concentration and macular pigment optical density (Arnold, Jentsch, Dawczynski, & Bohm, 2013; Kopsell & Lefsrud, 2006). Moreover, a recently published meta-analysis revealed that an increase in lutein and zeaxanthin intake (from natural food sources) might be protective against late AMD (Ma et al., 2012). Carotenoid contents in these vegetables are highly affected by genetic and environmental factors such as species, cultivars, growing conditions and postharvest handling (Rodriguez-Amaya, 2015). Additionally, food preparation such as trimming clearly reduces carotenoid contents, whereas mashing or moderate heating often increases carotenoid availability, very likely due to enhanced extractability following maceration of cells (Bohn et al., 2015). However, severe heat treatments such as baking or sterilization might cause significant losses.

Liberation including disintegration of the plant matrix and cellular breakage are prerequisites for carotenoid bio-accessibility and thus availability for intestinal absorption. *In vitro* methods simulating gastro-intestinal digestion offer a simple and fast approach to screen both the liberation and accessibility potential. So far, results have been difficult to compare between research groups due to differences in published models and/or parameters (Alminger et al., 2014). Within the COST action INFOGEST, digestion experts therefore consolidated parameters for simulating digestion of food and published a harmonised digestion protocol (Minekus et al., 2014). Thus, the aim of the present study was to pre-screen in a first step carotenoid liberation from green-leafy vegetables by applying this standardised *in vitro* protocol. Spinach and Asia salads (also known as Japanese Greens) were subjected to the commonly used domestic heat treatments, steaming and stir-frying. Additionally, the effect of particle size on carotenoid release was evaluated on spinach, whereas Asia salads of different cultivars were compared. *In vitro* accessibility results obtained in a follow-up study will be reported elsewhere (Eriksen et al., submitted).

2. Materials and methods

2.1. Samples and sample preparation

A batch of fresh baby leaf spinach (*Spinacia oleracea*) was purchased from a local retailer (Migros, Switzerland). It was either used as whole leafs (cut with a knife in strips of 3 cm to mimic whole leaf consumption) or finely ground in a cutter

* Corresponding author.
E-mail address: eva.arrigoni@agroscope.admin.ch (E. Arrigoni).

http://dx.doi.org/10.1016/j.foodchem.2016.02.033
0308-8146/

(La Moulinette DPA 1, Solingen, Germany) to prepare a puree. Commercial minced spinach was bought from the same retailer, but was manufactured from another batch. Asia salads were grown at the Agroscope Research Station in Wädenswil, Switzerland. Seeds of the cultivars Green Boy and Tatsoi Green (both *Brassica rapa* ssp. *chinensis*), Mibuna Early (*B. rapa* ssp. *nipposinica*) as well as Bloody Mary (*Brassica juncea*) were cultivated in pots (8 pots/cultivar, 6 seeds/pot) in the greenhouse at $\geqslant 5$ °C under daylight in February/March 2013. Plants were harvested after 6 weeks when they reached a height of 15–20 cm. Damaged or yellow leaves were removed and all plants were well mixed and cut with a knife in strips of 3 cm. All preparations were divided into aliquots for further experiments.

For analytical determinations, one aliquot was directly flushed with liquid nitrogen (Messer Schweiz AG, Lenzburg, Switzerland), divided into three portions, ground to a fine powder at −20 °C in the cutter, and stored in amber plastic bottles at −20 °C until analysis. For the experiments on domestic heat treatments, two aliquots were immediately transferred into plastic bags and shock-frozen in liquid nitrogen (below referred to as raw); two aliquots were steamed for 3 min at 100 °C, transferred into plastic bags and shock frozen (steamed); two aliquots were stir-fried with peanut oil (2.5 g/75 g vegetable) for 6 min under constant stirring and shock frozen (stir-fried). Whole leaves and spinach puree were processed in triplicates. All samples were stored at −20 °C in the dark until *in vitro* digestion experiments.

2.2. In vitro digestion

To evaluate carotenoid liberation, the *in vitro* digestion procedure based on the standardised COST Infogest protocol (Minekus et al., 2014) with electrolyte concentrations adapted from Kopf-Bolanz et al. (2012) was applied. Briefly, 5 g of sample was weighed into an amber screw-capped glass tube and incubated at pH 7.0 ± 0.2 for 10 min with 5 mL of simulated saliva fluid containing 1 mg of human saliva α-amylase (A1031, Sigma–Aldrich, Buchs, Switzerland). Next, 10 mL of simulated gastric fluid containing 3.9 mg of porcine gastric pepsin (P7012, Sigma–Aldrich) were added, pH was adjusted to 3.0 ± 0.2, the headspace flushed with nitrogen and the sample incubated for 2 h. Finally, after addition of 20 mL of simulated intestinal fluid containing 220 mg of porcine pancreatin and 334 mg of bile from bovine and ovine sources (P7545 and B8381, both Sigma–Aldrich), pH adjustment to 7.0 ± 0.2, and nitrogen flushing, the samples were incubated for another 2 h. All incubations were carried out at 37 °C in a shaking water bath at 90 strokes/min. Gastric and intestinal incubations as well as all following steps were done under red light. After incubation, the samples were immediately centrifuged twice for 10 min (4495 g, Haereus Multifuge 3RS+, Thermo Fischer Scientific, Reinach, Switzerland) to remove undigested solids and oil droplets, which are not considered available for absorption. 5 mL of the aqueous fraction including micelles were freeze-dried in the dark and immediately afterwards re-dissolved in extraction medium for carotenoid quantification. Each aliquot of domestic heat preparation was incubated twice.

2.3. Carotenoid quantification

The extraction procedure was performed under red light. Lutein and β-carotene contents were determined after extraction with methanol/acetone (1:1, v/v) containing 0.01% BHT as described previously (Reif, Arrigoni, Schärer, Nyström, & Hurrell, 2013). Starting materials were homogenised for 30 s in a Polytron blender at full speed under constant nitrogen supply followed by 30 min ultrasonic treatment, as were re-dissolved digestion supernatants. Aliquots of the extracts were filtered (Nylon syringe filters, pore size 0.22 μm) into brown UPLC-vials and immediately analysed.

UHPLC-PDA analysis was carried out based on the method described by Chauveau-Duriot, Doreau, Nozière, and Graulet (2010), modified by Eriksen et al. (submitted), by using an Acquity system (UPLC™, Waters Corporation, Milford, USA). In brief, separation was achieved on an Acquity UPLC column (HSS C18, 1.8 μm, 2.1 × 150 mm with a Vanguard pre-column HSS C18, 1.8 μm, 2.1 × 50 mm, Waters) with an ammonium acetate-acetonitrile-dichloromethane-methanol gradient. Quantification was based on external standards purchased from Carotenature (Lupsingen, Switzerland).

2.4. Data analysis

Carotenoid content in starting material is presented as mg carotenoid/100 g fresh material. *In vitro* carotenoid liberation was calculated as the fraction (%) of carotenoid released from the matrix to the total carotenoid content in the respective starting material. Data are presented as mean ± SD. Statistical difference for pairwise comparisons was calculated by student's *t* test, whereas for triple comparisons ANOVA followed by the Tukey–Kramer test was applied. *P* values <0.05 were considered significant.

3. Results and discussion

3.1. Carotenoid content

Table 1 reports the contents of lutein and β-carotene in the edible part of spinach and Asia salads. As for raw vegetables, carotenoid concentrations varied considerably between the two batches of spinach (as described in Section 2.1). This might be due to differences in cultivar, season, growing location, cultivation practise or industrial processing (Maiani et al., 2009). However, maturity is likely to play a key role, since minced spinach contained approx. 40% less lutein and β-carotene than whole leaves and puree which were produced from baby leaf spinach. This is in agreement with an earlier published investigation on New Zealand spinach showing a similar decrease from young to mature leaves (de Azevedo-Meleiro & Rodriguez-Amaya, 2005) and can be explained by changes in the leaf:rib (stem) ratio. Stems and ribs, which constitute a lower proportion in baby leaves, have been shown to be basically carotenoid-free (Reif, Arrigoni, Schärer et al., 2013). Similarly, stems of Tatsoi Green and Bloody Mary were more distinct than those of Mibuna Early and Green Boy, thus resulting in lower lutein and β-carotene contents. Taking differences in cultivars and agronomical practices into account, carotenoid contents of raw green leaves presented here, can be considered in good agreement with earlier published spinach (Kopsell & Lefsrud, 2006; Reif et al., 2012) and Asia salad data (Krumbein, Schonhof, & Schreiner, 2005; Reif, Arrigoni, Berger, Baumgartner, & Nyström, 2013).

Steaming affected the carotenoid content in the investigated vegetables differently (Table 1). No effect was seen for Asia salads and spinach puree. A rather small, but significant decrease due to steaming was observed for whole leaves, while the contents of both carotenoids in minced spinach were increased by more than 50%. Contradictory results have also been reported in the literature. Bunea et al. found no significant effects of boiling and steaming of spinach (Bunea et al., 2008), whereas Mazzeo et al. (2011) stated a significant increase in β-carotene due to steaming. Interestingly, frozen spinach had been used as starting material in the latter study as it was the case for our minced spinach. Whether industrial preparation (i.e. mincing, blanching and frozen storage) had an impact on carotenoid extractability after additional steaming in these samples, remains speculative. Interesting findings were obtained after stir-frying. Both lutein and β-carotene contents