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Comprehensive metabolomics to evaluate the impact of industrial processing on the phytochemical composition of vegetable purees

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ABSTRACT

The effects of conventional industrial processing steps on global phytochemical composition of broccoli, tomato and carrot purees were investigated by using a range of complementary targeted and untargeted metabolomics approaches including LC–PDA for vitamins, ^{1}H NMR for polar metabolites, accurate mass LC–QTOF MS for semi-polar metabolites, LC–MRM for oxylipins, and headspace GC–MS for volatile compounds. An initial exploratory experiment indicated that the order of blending and thermal treatments had the highest impact on the phytochemicals in the purees. This blending-heating order effect was investigated in more depth by performing alternate blending-heating sequences in triplicate on the same batches of broccoli, tomato and carrot. For each vegetable and particularly in broccoli, a large proportion of the metabolites detected in the purees was significantly influenced by the blending-heating order, amongst which were potential health-related phytochemicals and flavour compounds like vitamins C and E, carotenoids, flavonoids, glucosinolates and oxylipins. Our metabolomics data indicates that during processing the activity of a series of endogenous plant enzymes, such as lipoxygenases, peroxidases and glycosidases, including myrosinase in broccoli, is key to the final metabolite composition and related quality of the purees.

1. Introduction

A high intake of fruit and vegetables is recommended by health authorities worldwide due to a potential relationship with a reduced risk of cancer and cardiovascular diseases (Crowe et al., 2011; Leenders et al., 2013). Led by the consumers demand for more natural and healthier products, the food industry aims to (re)design manufacturing processes for improved nutritional quality of products based on fruit and vegetables. Carrot, tomato and broccoli are good sources of antioxidant vitamins (Steinmetz & Potter, 1996), next to many other phytochemicals that are potentially healthy for human, like various flavonoids and phenylpropanoids (Moco et al., 2006; Vallejo, Tomas-Barberan, & Ferreres, 2004). Broccoli also contains considerable amounts of glucosinolates, a group of phytochemicals specifically found in brassica vegetables (Moreno, Carvajal, Lopez-Berenguer, & Garcia-Viguera, 2006).

During food manufacture phytochemicals may undergo thermal degradation, air exposure due to disruption of plant tissues/cells and endogenous enzymatic activity. For example, vitamin C can be easily lost, especially during cooking, due to its sensitivity to heat (Francisco, Velasco, Moreno, Garcia-Viguera, & Cartea, 2010). Semi-polar phytonutrients like flavonoids and glucosinolates can also be lost upon heating vegetables (Aires, Carvalho, & Rosa, 2012; Francisco et al., 2010). In contrast, there is generally a relatively high retention of hydrophobic compounds such as β-carotene during thermal treatments (Svelander, Lopez-Sanchez, Pudney, Schumm, & Alminger, 2011).

Furthermore, not only the retention but also the bioaccessibility of health related compounds can be influenced by

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processing conditions due to the different microstructures which can be generated (Parada & Aguilera, 2007). For example, the *in vitro* bio-accessibility of lycopene from tomato can be increased by thermal and mechanical processing, as compared to unprocessed fruit (Tiback et al., 2009). Likewise, it has been shown that shear treatments to disrupt plant tissues/cells, such as high pressure homogenisation (HPH), can increase the bio-accessibility of carotenes from both tomato and carrot (Svelander et al., 2011). Thus, food processing may be optimised to enhance both the content and the bio-accessibility of phytonutrients present in final food products based on fruit and vegetables (Sanchez-Campillo et al., 2012).

It is still a considerable challenge for industries to design food manufacturing processes which provide products with the desired balance between nutritional and sensorial quality. A bottleneck in the design of such processes is the lack of insight into the impact of processing steps on the overall metabolite profile of fruits and vegetables. Metabolomics is a powerful tool to generate comprehensive insights into the metabolic composition of crude extracts and is increasingly being applied in food science (Beleggia et al., 2011; Capanoglu, Beekwilder, Boyacioglu, Hall, & De Vos, 2008; Hall, Brouwer, & Fitzgerald, 2008; McGhie & Rowan, 2012). A number of targeted metabolomics studies have been carried out to evaluate the impact of different processing conditions and steps on a specific class of phytochemicals, such as carotenoids in paprika paste (Schweiggert, Kurz, Schieber, & Carle, 2007) and glucosinolates in broccoli (Vallejo, Tomas-Barberan, & Garcia-Viguera, 2002), whereas large-scale untargeted metabolomics approaches have been applied to determine the global changes occurring upon processing of, for instance, tomatoes towards tomato paste (Capanoglu et al., 2008) and grain towards pasta (Beleggia et al., 2011).

In the present study, the effect of changing the order of thermal and mechanical processing steps on the phytonutrient profiles of carrot, tomato and broccoli purees was investigated, using a comprehensive metabolomics approach. Our objective was to evaluate the effect of different processing conditions on the phytonutrient profiles of the resulting vegetable purees. Experiments were therefore carried out on the same batch of material, to exclude biological variation between starting materials. We deployed both targeted analyses platforms, focussed on known health-related antioxidants, such as carotenes and vitamins C and E, and oxidised lipids, as well as a suite of complementary untargeted metabolomics platforms, including ^{1}H NMR for polar metabolites, accurate mass LC–QTOF MS for semi-polar metabolites and headspace-GC–MS for volatile compounds.

2. Material and methods

2.1. Materials

Three plant materials were selected due to the fact that they are major crops and they are present in many plant-based food products: carrots (*Daucus carota*) as an example of a root vegetable, broccoli (*Brassica oleracea*) as a stem vegetable and red ripe tomatoes (*Solanum lycopersicum*) as a fruity vegetable. They were purchased from a local supplier (Hofland, The Netherlands) and immediately processed.

The origin of all the chemicals and standards used in the various analytical methods are described in the related references. Sulforaphane was purchased from Sigma Aldrich.

2.2. Processing conditions

Carrot, broccoli and tomato were washed and cut into pieces of approximately 2 cm in size. The carrots were peeled prior to washing. The large stems of the broccoli and the cores of the tomato fruits were removed. Per vegetable, the pieces were randomly mixed into a single batch, of approximately 2 kg, to reduce variability from individual specimens and this batch was then divided into two sets, which were either first mechanically disrupted using a kitchen blender and then heated (B-T samples) or first heated and then blended (T-B samples). Pieces were mixed with deionised water, to facilitate the blending and homogenisation steps, in a ratio vegetable: water of 1:1 for carrot and broccoli, and 9:1 for tomato in view of the high amount of free water present in this fruit. One set of vegetable pieces (B-T) was firstly mechanically disrupted using a blender (Model CBT500E, Cuisinart®, East Windsor, NJ, USA) at maximum speed for 3 min. A sample of each of the resulting slurries was taken as the non-heated control (blended only; B) and the remainder was heated using a water bath. In the preliminary experiment we applied two conditions: 70 ± 5 °C for 10 min as a mild blanching (T1) and 90 ± 5 °C for 40 min as a more extreme cooking step (T2). The samples were covered with aluminium foil to prevent evaporation, as it was determined by weighing the samples before and after heating. The second set of pieces (T-B) was first heated in the same amount of water as the first set, followed by blending. The temperature in the core of the pieces was monitored using two thermocouples (manufactured in house) attached to a data logger. The samples were gently stirred during heating. In the exploratory experiment we also included high pressure homogenisation as a more severe mechanical treatment (60 MPa, Panda 2 k high pressure homogeniser, Niro Soavi, Parma, Italy). The processing conditions studied are representative of those thermal and shear treatments currently used in food industry to produce liquid and semi-liquid plant-based food products. Suppl. Fig. S1 summarises the combinations of the different processes.

For the large-scale comparative metabolomics study, both B-T2 and T2-B processing conditions were repeated 3 times on the same batch of raw material, in order to specifically focus on the effects of the differential order of processing and avoid inference from biological variation.

Irrespective of processing conditions, the resulting carrot, broccoli and tomato purees had a pH of 5.9, 6.2, and 4.3, respectively. Immediately after processing, the samples were frozen in liquid N_2 and ground to a fine powder, which was stored at −80 °C and kept frozen up to extraction into solvent for metabolite analyses.

2.3. Targeted analyses of vitamin C, sulforaphane and lipid-soluble isoprenoids

From each sample, 0.5 g frozen powder (FW) was weighed for extraction of lipid soluble isoprenoid compounds, including carotenoids, tocopherols and chlorophylls. Extraction and analysis was performed essentially as described previously (Capanoglu et al., 2008), using 2.5 ml methanol, 2 ml chloroform containing 0.1% BHT as antioxidant and 2.5 ml of 50 mM Tris-buffer pH 7.5. Sudan 1 (27 μg) was used as internal standard. Puree samples were extracted with chloroform three times, thereby collecting all visible pigments from the samples. Chloroform fractions were pooled and dried under N_2 gas, re-dissolved in 0.5 ml of ethylacetate and subsequently analysed by HPLC equipped with both a photodiode array detector (PDA; 240–600 nm) for the analysis of carotenoids and chlorophylls, and a fluorescence detector for the analyses of tocopherols. Vitamin C (ascorbic acid) was extracted using metaphosphoric acid and analysed by HPLC-PDA (Capanoglu et al., 2008). Sulforaphane was analysed by GC–MS after extraction with dichloromethane (Chiang, Pusateri, & Leitz, 1998). Authentic standards were used to construct calibration curves for compound quantification. Variation between replicate extractions and