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Influence of postharvest UV-B treatment and fermentation on secondary plant compounds in white cabbage leaves

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ABSTRACT

The influence of postharvest UV-B on its own and in combination with fermentation (e.g. sauerkraut production) on formation and degradation of bioactive compounds was investigated in white cabbage, processed according to traditional Chinese fermentation methods. The pattern of polyphenols was affected by postharvest UV-B: Newly formed coumaroylglycoside, feruloylglycoside, caffeoylglycoside (up to 1 mg/g dry matter; 4 days) and quercetintriglycoside (0.4–0.5 mg/g dm; 4 days) might be related to postharvest increase in enzyme activity in the biosynthesis. Decreasing contents were observed for the glucosinolates glucobrassicin and 4-methoxyglucobrassicin, but the formation of the degradation products dihydroascorbigen and dihydro-4-methoxyascorbigen, which might be related to cell shrinking as mechanical damage. Fermentation resulted in deglycosidation of hydroxycinnamic acids. Newly generated cinnamic acid from coumaric acid aglycone was detected in fermented plant material combined with UV-B (50 μg/g). Glucosinolates and dihydroascorbigens were completely degraded. This study shows exemplary UV-B as a supplemental step to improve the nutritional quality of processed plants.

1. Introduction

The health effects and bioactive capacity of foods derived from plants depend highly on post-harvest processing steps, such as storage, cutting, heating or fermentation. Postharvest treatments are often necessary to improve the storage and shelf life of vegetables, to avoid sensory disadvantages during storage, or to increase the nutritional values and palatability. The postharvest increase of bioactive compounds with nutraceutical activity in plants by applying postharvest abiotic stresses, such as wounding, temperature or light exposure (e.g. sun or UV light) is an important step to improve the nutritional value of fresh fruits and vegetables, as already mentioned by Cisneros-Zevallos (2003).

White cabbage (*Brassica oleracea* convar. *capitata* var. *alba*) is especially cultivated in Germany and widely used in processed food like coleslaw or traditional sauerkraut (fermented cabbage). Their nutritional relevance results from their high mineral, vitamin and dietary fibre content (Jahangir, Kim, Choi, & Verpoorte, 2009). White cabbage is also an important vegetable in the human diet mainly due to their contents of bioactive compounds. These compounds, such as polyphenols, glucosinolates, isothiocyanates, acorbigen or ascorbic acid, show a great abundance in *Brassica* vegetables and exert beneficial effects on human health. Due to these bioactive metabolites it is well known that *Brassica* vegetables and their extracts exhibit antioxidant activities, a decrease in oxidative damages, cardiovascular protective effects and inhibitory activities against chronic diseases like cancer (Jahangir et al., 2009).

However, Heimler, Vignolini, Dini, Vincieri, and Romani (2006) reported that a phenolic acid content in white cabbages was about 0.07 mg/g dry weight and the content of flavonoids was about 2.77 mg/g dw, determined by HPLC. Jahangir et al. (2009) reviewed the composition of different *Brassica* vegetables and reported the lowest flavonoid content for white cabbages (approx. in sum 60 μg/g fresh weight). Information on the polyphenolic contents in white cabbages and their structure elucidation (e.g. by HPLC and mass spectrometry) is scarce in literature and the main reported analytical methods are less precise photometric methods (e.g. Heimler et al., 2006; Kusznierewicz et al., 2008).

The major glucosinolates in white cabbages are glucobrassicin (precursor of ascorbigen), sinigrin, or glucoiberin as reported by Kusznierewicz et al. (2008) and Martinez-Villaluenga et al. (2009). Jahangir et al. (2009) reviewed contents of approx. 3.4 mg/g dw for sinigrin, 2.3 mg/g dw for glucoiberin and 1.3 mg/g dw for glucobrassicin. Sarikamis, Balkaya, and Yanmaz (2009) described glucobrassicin as the major glucosinolate in white cabbage heads.

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In general, white cabbage heads are cultivated in an open field. Only the minor part of the leaves of round compact cabbages is exposed to sun light and secondary plant compound formation, such as polyphenols, are only influenced in the outer leaves by UV-B radiation. However, the outer leaves carry no weight in food production, such as coleslaw or sauerkraut. In addition, in most cases, the outer leaves will be discarded prior to cabbage processing and treated as waste as described by Nilnakara, Chiewchan, and Devahastin (2009). However, cabbage processing sometimes includes further postharvest processing steps like sun exposure ('plant withering' before fermentation) as used for the traditional Chinese cabbage fermentation (also named 'pickling' of headless cabbages like pak choi), and this might influence the contents of bioactive compounds (Harbaum, Hubbermann, Zhu, & Schwarz, 2008).

The use of UV-B-light as a postharvest treatment (e.g. during storage) was investigated in previous studies in order to increase bioactive compounds, polyphenols, respectively: jaceidin in spinach, kaempferolglycoside in radish sprouts, or apigeninglycosides in parsley (Kanazawa, Hashimoto, Yoshida, Sungwon, & Fukuda, 2012), 5-O-caffeoylquinic acid in carrots (Avena-Bustillos et al., 2012), or ascorbic acid and carotenoids in tomato fruits (Castagna et al., 2013). The possibility to influence the phenolic content in plant materials by UV-B irradiation and their ability to protect against UV radiation is already known. UV radiation induces pre- and postharvest biological stress and the formation of polyphenols (Agati & Tattini, 2010; Hagen et al., 2007; Harbaum-Piayda et al., 2010). Avena-Bustillos et al. (2012) already mentioned that postharvest UV-B treatment has the potential to enhance the nutritional value of carrots. The enhancement of hydroxycinnamic acid contents is the consequence of an increase in enzyme activities, especially phenylalanine ammonia-lyase (PAL) (a key enzyme in the phenyl propanoid pathway in plants) as a biological response. Furthermore, Mewis et al. (2012) reported on UV-B-induction as an environmental factor of glucosinolates in broccoli sprouts.

UV radiation is also known as a food preserving technology (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010). For example, the application of partially ionised plasma for food sterilisation and inactivation of microorganisms in combination with UV irradiation, photons and radicals, is a mild technology at low and ambient temperatures (Moisan et al., 2002; Philip et al., 2002). Grzegorzewski, Rohn, Kroh, Geyer, and Schlüter (2010) reported on an increase of polyphenols by plasma application on lamb's lettuce after 120 s, but shorter times did not lead to significant changes. Rodov, Tietel, Vinokur, Horev, and Eshel (2010) reported on the postharvest physiological stimulation of flavonol enrichment in peeled onions due to the application of UV-light (UV-A, UV-B and UV-C) and the simultaneous decontamination of microorganisms on their surface.

Further, the postharvest microbial fermentation process is commonly used to preserve vegetables, for example cabbages (sauerkraut), olives, cucumber, eggplants, caper berries, or onions (Bisakowski, Atwal, Gardner, & Champagne, 2007; Rodríguez et al., 2009). Sauerkraut is prepared by shredding white cabbage leaves, salting, and placing in a vessel under pressure. In the case of the traditional Chinese fermentation procedure ("pickling"), salted cabbage plants are first withered in the sun (potential UV exposition for two days) prior to kneading, and then stored under pressure (Harbaum et al., 2008). Microorganism activities are responsible for the decrease in pH from approx. 7 to 4 during fermentation due to the production of different acids like lactic acid, propionic acid, and acetic acid (Trail, Fleming, Young, & McFeeters, 1996). The microbial population in the fresh cabbage before fermentation influences the quality of the sauerkraut (e.g. lactobacillus as a predominant microorganism or pathogenic microorganism, such as *Escherichia coli* and *Listeria monocytogenes*) (Peñas, Frias, Gomez, & Vidal-Valverde, 2010). Therefore, pre-fermenting treatments, such as UV-B, might influence the shelf-life of sauerkraut.

Structural changes in the phenolic pattern by cabbage fermentation have already been presented in previous studies for the Chinese cabbage pak choi (Harbaum et al., 2008). The structural changes of polyphenols might be caused by the cleavage of ester bonds or the split-off of functional groups by microorganisms, as reported for ferulic acid or *p*-coumaric acid (Cavin et al., 1997; Rodríguez et al., 2008). However, it was recently reviewed that the occurrence and content of food phenolics also influence the growth and viability of the *lactobacilli* species (Rodríguez et al., 2009).

Overall, the possibility to influence bioactive plant compounds and thus the nutritional value of foods by postharvest processes is of high interest to the food industry. In our study, white cabbage, or rather sauerkraut (fermented), served as a model plant with a low concentration of bioactive compounds, especially polyphenols. A profiling approach will be carried out for a detailed structural elucidation and quantification of polyphenols (phenolic acids and flavonoids) and elected glucosinolates by HPLC–DAD–MS. We are further interested in changes of their contents and the formation of known or newly identified degradation products by postharvest UV-B irradiation and fermentation. Therefore, the present study deals with the investigation on the effect of postharvest UV-B on its own on bioactive compounds. Further, this study will demonstrate the possibility to insert the step of UV-B treatment in existing postharvest processes, e.g. cabbage fermentation. The combination of standardized UV-B irradiation on bioactive compounds prior to fermentation was investigated according to the sun withering step, used for traditional Chinese cabbage fermentation, as described above (e.g. pak choi and Chinese leaf mustard; Harbaum et al., 2008). The results will be also discussed in a biochemical context.

2. Materials and methods

2.1. Chemicals

Acetonitrile (HPLC-grade, Fisher Scientific), ascorbigen (APIN chemicals), *t*-cinnamic acid (Merck), caffeic acid (Carl Roth GmbH), *p*-coumaric acid (Sigma), formic acid (Carl Roth GmbH), ferulic acid (Carl Roth GmbH), glucobrassicin (Phytolab), methanol (HPLC-grade, Fisher Scientific), oxalic acid dehydrate (Carl Roth GmbH), *m*-phosphoric acid (Fluka), sinapic acid (Carl Roth GmbH), and trifluoroacetic acid (Carl Roth GmbH) were utilized.

2.2. Plant material

Round white cabbage was selected as a model plant, due to low concentration of cinnamic acids and the absence of flavonoids. The variety 'Lennox' was obtained from a local market in October 2010 for the storage experiment under UV-B, and in January 2011 for the fermentation experiment.

2.3. UV-B treatment (experiment 1)

The leaf layers of three cabbage heads were separated into outer, middle and inner leaves. Between each layer, three leaves were discarded to enable a distinct separation of the different layers.

Cabbage leaves were randomized and one part was stored under UV-B light (0.3–0.4 W/m^2, 290–315 nm, 12 h per day) at 4 °C and the other part stored completely in the dark at 4 °C. Sampling was done in triplicate after 2, 4, and 7 days (7 days only for stored leaves in the dark). After sampling the plant material was freeze-dried, ball-milled and stored until further analysis.