Food Chemistry 145 (2014) 264–271

Contents lists available at ScienceDirect

Food Chemistry

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

Optimization of a blanching step to maximize sulforaphane synthesis in broccoli florets

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ARTICLE INFO

Article history:
Received 16 May 2013
Received in revised form 9 August 2013
Accepted 14 August 2013
Available online 27 August 2013

Keywords:
Glucosinolates
Myrosinase
Glucoraphanin
Hydrothermal treatment

ABSTRACT

A blanching step was designed to favor sulforaphane synthesis in broccoli. Blanching was optimised through a central composite design, and the effects of temperature (50–70 °C) and immersion time in water (5–15 min) on the content of total glucosinolates, glucoraphanin, sulforaphane, and myrosinase activity were determined. Results were analysed by ANOVA and the optimal condition was determined through response surface methodology. Temperature between 50 and 60 °C significantly increased sulforaphane content ($p < 0.05$), whilst blanching at 70 and 74 °C diminished significantly this content, compared to fresh broccoli. The optimal blanching conditions given by the statistical model were immersion in water at 57 °C for 13 min; coinciding with the minimum glucosinolates and glucoraphanin content, and with the maximum myrosinase activity. In the optimal conditions, the predicted response of 4.0 μmol sulforaphane/g dry matter was confirmed experimentally. This value represents a 237% increase with respect to the fresh vegetable.

1. Introduction

Glucosinolates (GSL) are secondary metabolites that are found in a relatively high concentration in Brassicaceae plants, such as cauliflower, Brussels sprouts and broccoli, amongst others. Epidemiological studies demonstrated that a Brassicaceae-rich diet reduces the risk of developing some types of cancer, most likely due to the GSL found in these vegetables (Latté, Appel, & Lampen, 2011; Manchali, Chidambara Murthy, & Patil, 2012). Recently, great attention has been set on broccoli (*Brassica oleracea* var. *italica*) due to its remarkable healthy effects (Mahn & Reyes, 2012). In some broccoli cultivars, the most abundant GSL is glucoraphanin, whose hydrolysis by the action of myrosinase (thioglucoside glucohydrolase; EC 3.2.1.147) yields sulforaphane, an isothiocyanate recognised as the most powerful anti-cancer compound naturally found in food (Matusheski, Juvik, & Jeffery, 2004). However, sulforaphane is not the only hydrolysis product of glucoraphanin, since the reaction strongly depends on the chemical conditions (pH, temperature, presence of cations and ascorbic acid) (Ludikhuyze, Rodrigo, & Hendrickx, 2000). In addition to sulforaphane, other non bioactive and even toxic compounds are formed, namely nitriles, and to a lesser extent thiocyanates and epithionitriles. Fig. 1 shows a schematic representation of the hydrolysis of glucoraphanin by the action of myrosinase. Sulforaphane synthesis is favored by high temperature (up to 70 °C) and neutral pH, whereas nitrile formation occurs at low temperatures (below 50 °C) and acid pH (Howard, Jeffery, Wallig, & Klein, 1997; Mahn & Reyes, 2012). Nitrile formation is favored at high ferrous ion concentrations (>0.01 mM) and by the presence of nitrile – specifier proteins (NSPs) that promote nitrile formation at physiological pH values (Wittstock & Burow, 2010) Besides, nitrile formation is triggered by the action of the epithiospecifier protein (ESP) (Williams, Critchley, Pun, Nottingham, & O'Hare, 2008), which is much more thermo labile than myrosinase (Matusheski et al., 2004).

On the other hand, in the vegetable tissue, myrosinase is found in specialised myrosin cells (Shapiro, Fahey, Wade, Stephenson, & Talalay, 2001), and accordingly, in the intact vegetable myrosinase is physically segregated from its substrate, glucoraphanin. Therefore the hydrolysis proceeds only when the tissue is damaged, either by mechanical action, harvesting, processing or mastication, allowing enzyme and substrate to come together. Given that broccoli is mainly consumed as a processed food (cut, blanched, cooked, frozen, etc.), and considering that processing usually impairs quality and promotes the loss of bioactive compounds, in this work we postulate that it is possible to increase significantly the sulforaphane content in processed broccoli by managing adequately the blanching conditions.

Some studies have shown that cooking significantly affects the content of total glucosinolates and its hydrolysis products, as well as myrosinase activity. Cieślik, Leszczyńska, Filipiak-Florkiewicz, Sikora, and Pisulewski (2007) reported that blanching and boiling

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http://dx.doi.org/10.1016/j.foodchem.2013.08.053

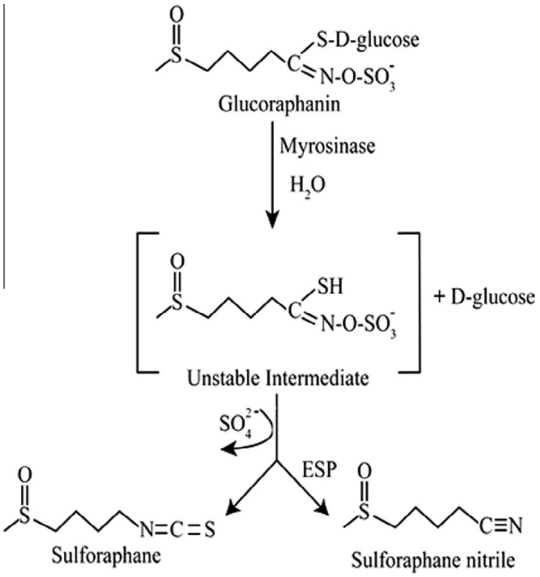

Fig. 1. Schematic representation of the hydrolysis of glucoraphanin mediated by myrosinase.

Brassicaceae vegetables produced significant loss of glucosinolates, varying from 2.7% and 30.0% in blanched vegetables and from 35.3% and 72.4% in boiled vegetables, with respect to the fresh material. Gliszczyńska-Świgło et al. (2001) informed the loss of myrosinase activity in cabbage subjected to microwave cooking for 2 min or steaming for 7 min.

Few studies have focused on the effect of hydrothermal treatments on sulforaphane synthesis in broccoli Howard et al. (1997) reported sulforaphane loss when broccoli was blanched or microwave cooked. Jones, Frisina, Winkler, Imsic, and Tomkins (2010) found a 90–98% reduction of sulforaphane content in broccoli (cvs Marathon and Broster) subjected to boiling, steaming and microwave cooking. Martínez-Hernández, Artés-Hernández, Gómez, and Artés (2013) detected no sulforaphane formation in broccoli subjected to different types of cooking (steaming, microwave, low pressure, etc.). The same authors reported that only in grilled broccoli a 0.15% sulforaphane retention was achieved. The results were attributed to the complete loss of myrosinase activity. On the contrary, Wang, Farnham, and Jeffery (2012a) found that short thermal treatment of broccoli, namely microwave and boiling for 0.5–0.75 min and steam cooking for 1–3 min, significantly increased sulforaphane content compared with fresh broccoli. Additionally, Matusheski et al. (2004) reported that a short hydrothermal treatment, at 50–60 °C during 10 min, inactivates ESP and kept the activity of myrosinase. Accordingly it should be possible to design a blanching step that inactivates ESP and enhances myrosinase activity in order to produce a blanched broccoli rich in sulforaphane.

Table 1
Experimental matrix.

Treatment	Temperature (°C)	Time (min)
T1	46	10
T2	50	5
T3	50	15
T4	60	3
T5	60	10
T6	60	17
T7	70	5
T8	70	15
T9	74	10

Currently there is no study about the effect of blanching conditions that considers the effects of temperature and time on the sulforaphane content in broccoli. The aim of this work was to investigate the effect of blanching conditions (temperature and immersion time) on the content of total glucosinolates, glucoraphanin, sulforaphane and myrosinase activity in broccoli, and to determine the blanching conditions that maximizes sulforaphane synthesis.

2. Materials and methods

2.1. Experimental design and statistical analyses

A central composite design of uniform precision was used, with two experimental factors: temperature (50 and 70 °C) and time (5 and 15 min), four axial points and five central points. The response variables were the content of total GSL, sulforaphane, glucoraphanin, and myrosinase activity. Table 1 shows the experimental matrix in standard order. The statistical effect of the experimental factors on the responses was determined by the response surface methodology, using a second order polynomial model to describe the experimental behavior (Eq. (1)).

$$\hat{Y} = \beta_0 + \sum_{i=1}^{k}\beta_i\chi_i + \sum_{i=1}^{k}\beta_{ii}\chi_i^2 + \sum\sum_{i<j=1}^{k}\beta_{ij}\chi_i\chi_j \quad (1)$$

where $\hat{Y}$ is the predicted value of the response; β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for interception, linear, quadratic and interaction effects, respectively, k is the number of independent parameters ($k = 2$ in this study), and X_i, X_j are the coded levels of the experimental conditions. Analysis of variance (ANOVA) was applied to determine significant effects of temperature and time on the responses. The model quality was assessed by the determination coefficients (R^2) and root mean of square error (RMSE). This study design was analysed and three-dimensional response surface plots were drawn using JMP 9.0.1 software (SAS Institute).

2.2. Plant material

Broccoli (*Brassica oleracea* cv. Avenger) heads (three days from harvesting) were purchased at the local market (Santiago, Chile) to a unique supplier. Broccoli florets were cut in 5-cm length and 0.7–0.9 cm width (stem) before blanching.

2.3. Blanching

In each experimental run, 300 g of cut broccoli were immersed in 1.5 L deionised water contained in a thermostatic bath (Stuart, United Kingdom, Great Britain), at different temperatures for different time periods, as stated by the statistical design. After blanching, broccoli pieces were immediately put in a water ice bath and then they were stored at −20 °C until analysis.

2.4. Analytical determinations

2.4.1. Moisture content

The moisture content of fresh and blanched samples was determined in a vacuum oven until constant weight according to AOAC 920.151. All determinations were made in triplicate.

2.4.2. Total GSL content

Extraction and quantitative analysis of total GSL were performed according to Hsu et al. (2011) with some modifications. Fresh and blanched vegetable samples were pulverized with liquid nitrogen in a mortar, until obtaining a homogeneous meal. 280 μL acidised methanol (40% methanol and 0.5% acetic acid; to prevent