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Study on degradation kinetics of sulforaphane in broccoli extract

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ABSTRACT

The objective of this study was to investigate the thermal degradation kinetics of sulforaphane (SF) in broccoli extract at selected temperatures (60, 75, 82 and 100 °C) and pH values (2.2, 3.0, 4.0, 5.0 and 6.0). The results indicated that SF is unstable at high temperatures, but is more heat-stable when present in acidic food products. The degradation rate constants of SF in broccoli extract were lower than those obtained in purified SF. The thermal degradation of SF followed first-order reaction kinetics, and the rate constant increased with increase of temperature and pH values. The rate constant vs temperature relationships, which yield linear Arrhenius plots, were described by a simpler exponential equation, and a mathematical model was developed, using the steady-state kinetic parameters obtained to predict the retentions of SF at various pH values, heating times and temperatures.

1. Introduction

Epidemiological studies have shown that the consumption of cruciferous vegetables, such as broccoli and Brussels sprouts, is linked to reduced cancer risks (Verhoeven, Goldbohm, van Poppel, Verhagen, & van den Brandt, 1996). A great deal of research regarding cruciferous vegetables has focussed on sulforaphane (4-methylsulfinylbutyl isothiocyanate, SF), which is a hydrolysis product of glucoraphanin, the primary glucosinolate found in broccoli. SF is of interest since it primarily modulates the activities of phase II enzymes that convert carcinogens to inactive metabolites, thereby preventing them from interacting with DNA (Zhang, Kensler, Cho, Posner, & Talalay, 1994). SF can also inhibit histone deacetylase activity in human colorectal and prostate cancer cells, resulting in enhanced histone acetylation, derepression of *P21* and *Bax*, and induction of cell cycle arrest/apoptosis, leading to cancer prevention (Dashwood & Ho, 2007; Ho, Clarke, & Dashwood, 2009). In several studies, SF has been shown to lower the risks of various cancers, such as lung cancer, colorectal cancer, breast cancer, prostate cancer, prostate cancer and/or bladder cancer (Ciska & Pathak, 2004; Higdon, Delage, Williams, & Dashwood, 2007; Kristal & Lampe, 2002; Latté, Appel, & Lampen, 2011). These findings all raise the possibility that SF may be an effective substance for reducing the risk of cancer.

However, SF is not a stable compound, its stability is affected by pH, temperature, heating time and oxygen (Wu, Liang, Yuan, Wang, & Yan, 2010). To date, many studies of the degradation of SF, with respect to different conditions and systems, have been reported. Jin, Wang, Rosen, and Ho (1999) studied SF degradation in an aqueous solution at 50 and 100 °C, the major degradation product was a thiourea compound. The stability of SF to heat is enhanced by formation of a SF-hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion complex (Wu et al., 2010). Very recently, the degradation rates of pure SF and its HP-β-CD inclusion complex were studied by Wu, Mao, Mei, and Liu (2013), and the kinetic parameters of the degradation of pure SF and its HP-β-CD inclusion complex were described by the Arrhenius equation. However, there are concerns that the stability of SF may also be affected by some food components. It is therefore necessary to study the kinetic parameters of SF in crude broccoli extract.

In food research, the Arrhenius equation has been widely used as a means to quantify the effect of temperature on several chemical and biochemical reactions; then "energy of activation", E_a, can be calculated from the slope of the linear Arrhenius plot. However, Peleg, Normand, and Corradini (2012) argued that there are several problems involved with applying the Arrhenius model to food systems. For example, when the Arrhenius equation is used for modelling microbial inactivation or beverage sterilization, it is impossible to define a "mole" of bacterial cells or orange juice. Similar doubts exist for the coordinate compression in the Arrhenius plot; e.g., it makes a big difference if the temperature is 5 or 40 °C. Yet, this huge temperature range is transformed into the meager 0.0036–0.0032 K^{-1} range (Peleg et al., 2012). Attempts

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have therefore been made by a number of researchers to develop other models to replace the Arrhenius equation. For example, Sapru and Labuza (1993) attempted to replace the Arrhenius equation by the Williams–Landel–Ferry (WLF) model. Recently, Peleg et al. (2012) proposed a simpler exponential model without sacrificing the goodness of fit. In this study, we employed the exponential model to depict the degradation of SF in broccoli extract.

The aim of this kinetic study was to determine the kinetic parameters governing the thermal degradation reactions of SF, particularly with respect to the effects of temperature and pH value, which are the main factors influencing the degradation, to advance knowledge of the thermal stability of SF in broccoli extracts, and to establish mathematical models enabling the prediction of SF degradation in crude broccoli extract during storage and/or thermal processing.

2. Materials and methods

2.1. Materials and chemicals

Broccoli seed was kindly provided by Taizhou Academy of Agricultural Science. Distilled water was used throughout the study. Methanol (TEDIA, USA) was HPLC grade, and all other reagents were of analytical reagent grade and were purchased from Huadong Medicine Co. Ltd. (Hangzhou, China). Pure SF was purified as previously reported (Wu et al., 2013).

2.2. Extraction methods

50 g of broccoli seeds were ground in a Chinese herbal medicine grinder to produce seed meal; the seed meal was subsequently defatted with 400 ml of hexane in an incubator shaker for 3 h, after which the residual seed meal was allowed to dry in a fume hood. De-fatted seed meal was mixed with 100 ml of potassium phosphate buffer (0.05 M, pH 5.8) and 200 ml of ethyl acetate; the resulting mixture was agitated for 4 h and then 20 g sodium chloride were added and mixed thoroughly. The ethyl acetate layer was filtered and the residual paste was extracted (2 times) with equal volumes of ethyl acetate, which were combined and dried in a vacuum rotavapor. The residue was filtered through a 0.45 μm membrane for HPLC analysis. SF destruction may occur during the extraction procedure due to its instability; thus the extract procedure should be conducted at room temperature.

2.3. Kinetic modelling of SF in broccoli extract degradation

The effect of pH on SF in broccoli extract thermal stability was studied at five pH values (2.2, 3.0, 4.0, 5.0 and 6.0) and four temperatures (60, 75, 82 and 90 °C). The SF in broccoli extract degradation was studied by the method of Wu et al. (2013). Briefly, citrate–phosphate buffers were prepared to provide the specified pH situation, and SF was dissolved with 0.01 M citrate–phosphate buffer at each pH value to get concentrations of 0.12 mg/ml. 2 ml of SF solution was put into a plastic tube (3 ml total volume, Thermo Fisher Scientific Inc., USA). The sample tubes (6 tubes per each pH), covered with aluminium foil, were well capped to avoid evaporation and were placed in a thermostatic water bath (Beijing Era Beili Centrifuge Co. Ltd., China) preheated to a given temperature. At regular time intervals (1 h intervals), one tube of each pH was randomly taken from the water bath and rapidly cooled by plunging into an ice water bath (Hou, Qin, Zhang, Cui, & Ren, 2013). The SF contents of cooled tubes were analysed by HPLC. All experiments were done in triplicate. Parameters of the kinetic model included the reaction rate constant (k). The rate of SF degradation during heat processing can be modelled as Eq. (1):

$$dC/dt = -kC^n, \quad (1)$$

where C, t, k, and n represent the concentration of SF (mg/ml), the time (h), the reaction rate constant (h^{-1}) and the kinetic order of the reaction, respectively.

Furthermore, the temperature dependence of rate constant is simulated with an exponential model equation as follows (Peleg et al., 2012):

$$\ln\left[\frac{k(T)}{k(T_{ref})}\right] = c(T - T_{ref}). \quad (2)$$

Thus, the equation could be simplified to Eq. (3):

$$\ln\ k = cT - b, \quad (3)$$

where $k(T)$ is the reaction rate at temperature T in °C, T_{ref} is the reaction rate at a reference temperature $T_{reference}$ in °C, and c is a constant having $°C^{-1}$ units; b is also a constant.

2.4. The effect of ascorbic acid on the stability of SF

The heating process was according to the method 2.3, except that pure SF (0.12 mg/ml) was employed and various concentrations (0.005%, 0.01%, 0.04%, 0.07% and 0.10%) of ascorbic acid were added. The heating time was 6 h and heating temperature was 85 °C.

2.5. HPLC

The content of SF was analysed on a Waters e2695 HPLC system by the method of Wu et al. (2013). The column employed in our experiment was a ZORBAX Eclipse XDB-C18 (4.6 × 250 mm, 5 μm). The mobile phase consisted of 20% methanol in water, changing linearly over 10 min to 60% methanol, then increasing to 100% in 2 min and then maintained for 2 min to purge the column. The column oven temperature was set at 25 °C, the flow rate was 1.0 ml/min, and 10 μl samples were injected onto the column. SF was detected using a Waters 2489 detector at 241 nm.

2.6. Statistical analysis

All experiments were done in triplicates and the results were expressed as mean values. The errors of experimental data from the mean values were expressed as standard deviation, using the Microsoft Excel software for Mac 2011 and illustrated as error bars.

3. Results and discussion

3.1. Stability of extracted SF during heat treatment and under different acidic conditions

The effects of pH on the stability of SF are presented in Table 1. The results indicate that SF was quite stable at low pH values and temperatures. At pH 2.2, even with heating at 60 °C for 6 h, more than 95.1% of the SF was retained. However, the retention value decreased as the pH value and temperature increased. When the pH value increased to 6.0, even at the lowest tested temperature of 60 °C, 32.1% of the SF was lost in 6 h. After heating for 6 h at 90 °C and pH 6.0, only 6.0% of the SF remained (Table 1). In general, increasing the pH from 3.0 to 5.0 hastened the degradation of SF, suggesting that SF is unstable at high temperatures, particularly under high pH conditions. In other words, SF is more heat-stable when SF is used in acidic food products. Our results were in agreement with those of previous studies, which report that the amount of SF decreased slowly in acidic environment but suffered a more severe decrease in neutral or alkaline conditions (Wu et al., 2010).