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Short communications

Commercially produced frozen broccoli lacks the ability to form sulforaphane

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ABSTRACT

Sulforaphane is produced from the hydrolysis of the glucosinolate glucoraphanin in the presence of the endogenous enzyme myrosinase. Sulforaphane has been shown to provide cancer prevention through a number of mechanisms including the upregulation of detoxification enzymes and epigenetic changes. Optimal temperature and pH for sulforaphane formation from broccoli was determined. Sulforaphane formation was measured in three commercially frozen broccoli samples pre- and post-cooking. The results show that in these products, there was very little potential to form sulforaphane prior to cooking and essentially none after the recommended cooking method was performed. Research is needed towards improved processing methods.

1. Introduction

Fresh broccoli contains myrosinase, an enzyme which, in the presence of water, converts the glucosinolate glucoraphanin into the bioactive compound sulforaphane. Sulforaphane has been shown to be a potent cancer-preventative compound acting as a strong inducer of phase II detoxification enzymes (Zhang, Talalay, Cho, & Posner, 1992; Jeffery & Araya, 2009). Recent work has also shown that broccoli sprouts, which produce high concentrations of sulforaphane upon crushing, can assist in the management of inflammation caused by type 2 diabetes (Mirmiran, Bahadoran, Hosseinpanah, Keyzad, & Azizi, 2012). Blanching is a common practice used in frozen vegetable processing, where heat in the form of water or steam, is used to inactivate degradative enzymes to prolong the shelf life of the product (Andress & Harrison, 2006). The temperature and time used can vary, but most commonly loss of activity of the thermally stable enzyme peroxidase is used as an indication of blanching thoroughness (USDA, 1975). Use of the enzyme peroxidase as an indicator enzyme has come under criticism, since it does not play a direct role in degradation during frozen storage (Barrett & Theerakulkait, 1995). Also, when blanching to inactivate peroxidase, unnecessary nutrient and product degradation may occur (Lim, Velasco, Pangborn, & Whitaker, 1989). The broccoli enzyme myrosinase is not thermally stable, showing 90% degradation when held at 60 °C for 10 min (Van Eylen, Oey, Hendrickx, & Van Loey, 2007). Blanching

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Abbreviations: DCM, dichloromethane; BITC, benzyl isothiocyanate

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protocols following industrial practices can reach conditions that well exceed this time and temperature (Lund, 1977). Coupled with the cooking procedures that frozen broccoli undergoes, this process far exceeds the reported temperature stability of the broccoli enzyme myrosinase (activity determined using sinigrin as substrate). Therefore we hypothesized that commercial heat processing for frozen broccoli production would slow or prevent the formation of sulforaphane.

2. Materials and methods

Three brands of frozen broccoli were purchased from a local grocery store (Meijer). These were the only available broccoli products which were not mixed with a seasoning or sauce. Samples of each were then cooked per the manufacturer's instructions in an 1100 Watt microwave oven (Sharp Carrousel). Two frozen broccoli brands required that the product be in a covered bowl; the third, that the product be steamed in the bag. For comparison, samples from each product were heated in a bowl and separate samples were heated in steamer bags. Following cooking, the broccoli was frozen in liquid nitrogen, freeze dried and ground. In triplicate, ground broccoli (100 mg) was added to 1.5 mL distilled water, vortexed, and left to hydrolyze in the dark at room temperature for either 20 min or 24 h. The reaction was then stopped by heating the sample at 90 °C for 5 min in a water bath. The mixture was then centrifuged for 8 min at 14,000*g* and filtered through a 0.45 μm nylon syringe filter and immediately analyzed for sulforaphane formation.

2.1. *Temperature and pH dependence of broccoli hydrolysis*

Broccoli (*Brassica oleracea* L. cv. "Green Magic") was grown at the University of Illinois in 2009, harvested at commercial maturity and transported to the lab on ice for freeze drying within 2 h. Freeze dried broccoli (0.1 g) was hydrolyzed for 20 min in 1.5 mL of McIlvaine's buffer ranging in pH (2, 3, 4, 5, 6, 7, 8). The pH at which the maximum sulforaphane formation occurred was then used in a series of hydrolysis trials at different temperatures (4, 14, 25, 34, 44, 54 and 64 °C). Samples were hydrolyzed for 20 min at these temperatures and then analyzed for sulforaphane formation.

2.2. *Sulforaphane analysis*

An internal standard of benzyl isothiocyanate (BITC) was added to 0.5 mL broccoli hydrolysis supernatant and isothiocyanates were extracted into 0.5 mL dichloromethane (DCM) for analysis by gas chromatography. Using an Agilent model 7683B series auto sampler, 1 μL DCM extract was injected onto an Agilent 6890N gas chromatography system equipped with a single flame ionization detector (Agilent Technologies, Santa Clara, CA). Samples were separated using a 30 m × 0.32 mm J&W HP-5 capillary column (Agilent Technologies). After an initial hold at 40 °C for 2 min, the oven temperature was increased by 10 °C/min to 260 °C and held for 10 min. Injector temperature was 200 °C; detector temperature was 280 °C. Helium carrier gas flow rate was 25 mL/min. Using Statistical Analysis Software (SAS; Cary NC), data were compared by ANOVA followed by LSD ($\alpha = 0.05$) where differences were indicated. All chemicals were purchased from Sigma Chemical (St. Louis, MO) unless stated otherwise.

3. Results and discussion

The focus of this study was on the ability of myrosinase to catalyze the formation of sulforaphane within commercially-available frozen broccoli. This was characterized by first determining the optimum temperature and pH at which sulforaphane was formed over a 20 min time period. This period was chosen because substrate would not be limiting and therefore the quantity of product formed would be dependent on the activity of the myrosinase in the sample. In contrast, a sample hydrolyzed for 24 h provided excess time for substrate hydrolysis to be completed. This information gives insight into the stability of myrosinase under many conditions, including those that might be incurred during processing and handling – and therefore might affect the ability of myrosinase to form sulforaphane later. The optimum pH and temperature at which sulforaphane formation occurs in

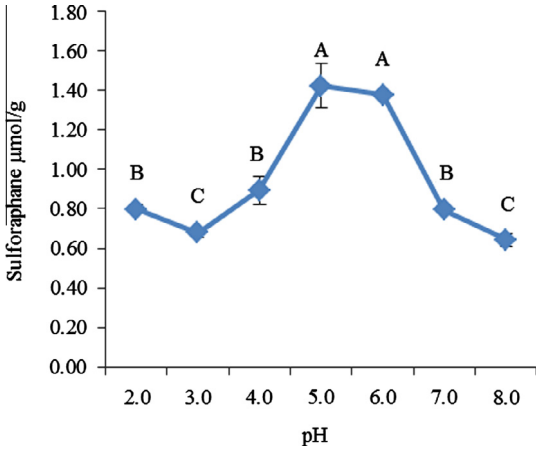

Fig. 1 – Effect of pH on sulforaphane formation during a 20 min broccoli hydrolysis at room temperature (25 °C). Different letters represent significantly different values ($p < 0.05$), data are given as mean ± SD, $n = 3$.

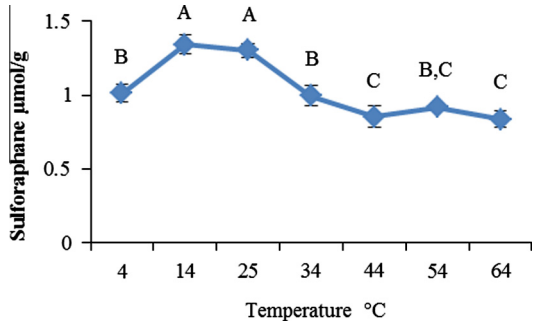

Fig. 2 – Effect of temperature on sulforaphane formation during a 20 min hydrolysis at pH 5. Different letters represent significantly different concentrations of sulforaphane ($p < 0.05$), data are given as mean ± SD, $n = 3$.