

LiquaDry Preliminary Report 9-14-10

Korry Hintze, PhD
Utah State University
Dept. Nutrition, Dietetics and Food Science
Logan, UT

Background

Epidemiological studies have implicated crucifer vegetable (such as broccoli) consumption as a positive modifier of cancer risk. In a cohort of 47,909 health professionals, Michaud et al., [1] found that the incidence of bladder cancer was decreased specifically by crucifer intake independent of total vegetable or fruit consumption. Similar reports exist for breast cancer risk [2] and prostate cancer [3, 4]. The mechanism for this decrease in carcinogenesis is mediated via upregulation of antioxidant and detoxification genes by isothiocyanates derived from cruciferous plants [5]. The primary isothiocyanate responsible for this increase is thought to be sulforaphane [6]. Sulforaphane is not found in crucifer plants per se but is derived by the hydrolysis of glucosinolates (GS) found in the plant. Glucosinolates are hydrolyzed by the plant enzyme myrosinase which is compartmentalized separately from GS. When the plant is masticated, myrosinase is released and the GS are hydrolyzed to either isothiocyanates (such as SF), nitriles or thiocyanates (See Figure 1).

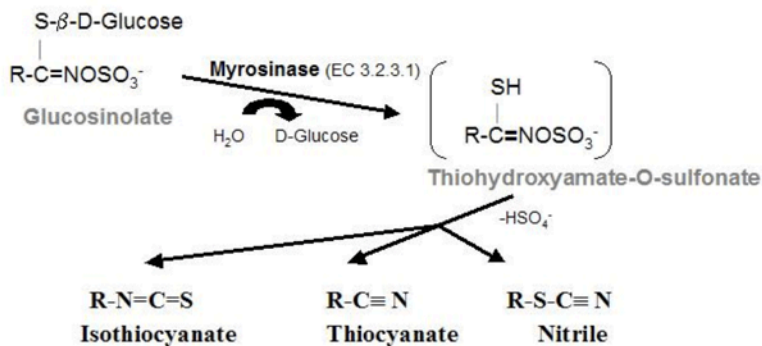


Figure 1. Glucosinolate Hydrolysis Products from Broccoli

Because both myrosinase and sulforaphane are heat labile, the anti-carcinogenic properties broccoli can be lost through improper cooking or processing. A study by Van Eylen and co-workers [7] found that myrosinase is completely denatured at 70 C (see figure 2). They also demonstrated that SF, that the anti-carcinogenic byproduct of the myrosinase/glucosinolate reaction, is completely denatured at 90 C and 50% of SF is denatured at 70 C. Commercial spray drying subjects botanicals to temperatures of up to 82 C.

Figure 2. Effect of Temperature on Myrosinase Stability. From Van Eylen et al., [7].

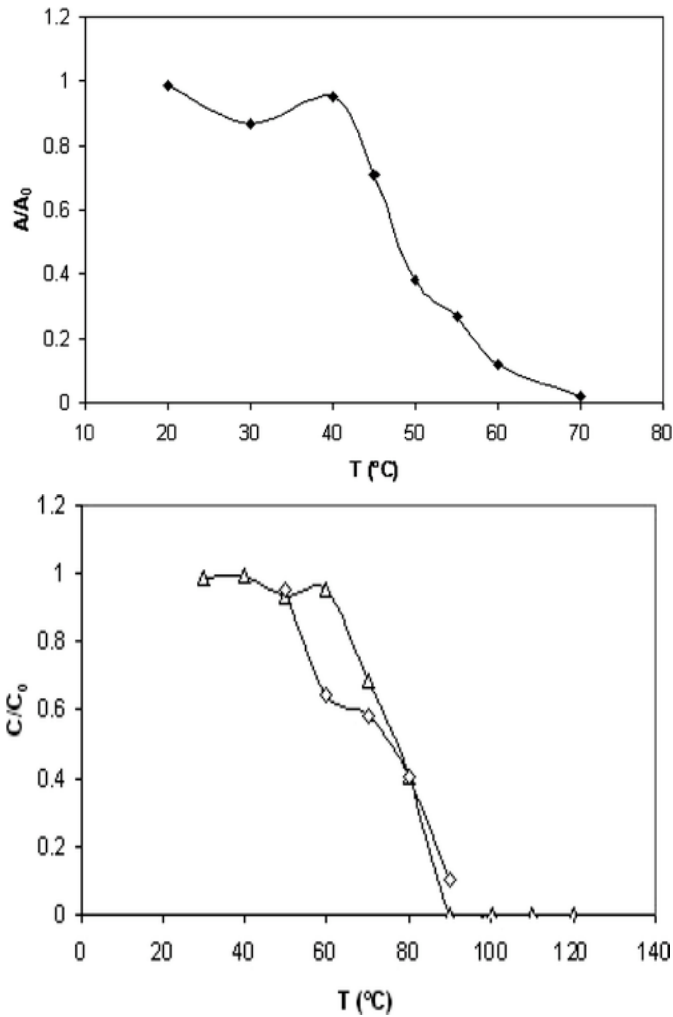


Figure 3. Effect of Temperature on Sulforaphane (Diamond) Stability. From Van Eylen et al., [7].

We hypothesized that the extreme processing method of spray drying results in broccoli powders devoid of sulforaphane and most (if not all) of the anti-carcinogenic benefit will be lost. Because Liquadry uses a dramatically less heat intensive drying process (~42 C) it is likely that resulting broccoli powders will contain significantly more sulforaphane and thus retain anti-carcinogenic properties. Therefore, the goal of the study was to compare antioxidant gene activation (anti-carcinogenic potential) of broccoli powders derived from spray drying and Liquadry processed broccoli powder.

Materials and Methods

Broccoli Processing

Broccoli slurry was made at room temperature with a homogenizer then frozen and aliquoted for drying at LiquaDry (low temperature drying) or Utah State University (high temperature spray drying). Broccoli slurry was thawed overnight and dried upon thawing. Broccoli slurry was dried using either the low temperature (42 C) proprietary, LiquaDry process or by standard spray drying. Standard spray drying was completed using a Lab Plant SD04 pilot scale spray drier; the inlet temperature was 150 C and outlet temperature was 90 C.

Antioxidant Gene Activation

Antioxidant gene activation potency of broccoli powders was determined by the method of Hintze et al., [9]. Hepa1c1c7 liver cells were grown on 12-well plates under standard cell culture conditions. After 24 h, cells were transfected with pGL3 luciferase reporter constructs containing the entire quinone reductase gene promoter along with pRL-SV40, an expression vector for renilla luciferase to be used as an internal control. Induction of the quinone reductase promoter is a well-established marker of antioxidant gene activation by botanicals [9-13]. Twenty-four hours after transfection, media was changed and dried broccoli extracts were added to the culture media. In preliminary experiments, it was determined that broccoli extracts added to the culture media in excess of 1 mg/ml were toxic so LiquaDry and spray dried extracts were added to the culture media at the following concentrations: 0.0001, 0.001, 0.01, 0.1 and 1 mg/ml. Twenty-four hours after experimental treatments are added, cells were harvested and assayed for luciferase activity using the Dual Luciferase Assay Kit (Promega) by luminometry to determine antioxidant gene induction. Induction of quinone reductase by extracts was compared to control cells with no extract or positive control media that contained 4 uM of purified sulforaphane.

Results

As expected, treatment of cells with 4 uM sulforaphane significantly increased ($P < 0.05$) quinone reductase induction over controls (Figure 4). Spray dried extracts did not increase quinone reductase induction over controls regardless of concentration. Conversely, low temperature dried, LiquaDry extracts increased quinone reductase when supplied at 0.1 and 1 mg/ml ($P < 0.05$).

These data suggest that high temperatures associated with standard spray drying are detrimental to broccoli bioactivity and that lower drying temperatures are essential to maintain the anticarcinogenic effects associated with broccoli intake. This observation is in agreement with work by Van Eylen et al., [7] who demonstrated that sulforaphane is labile at temperatures over 80 C but is stable at temperatures used in the low temperature drying (Figure 3). This work suggests that broccoli extracts manufactured by traditional spray drying may not provide anticancer benefits associated with broccoli consumption compared to extracts dried at lower temperatures.

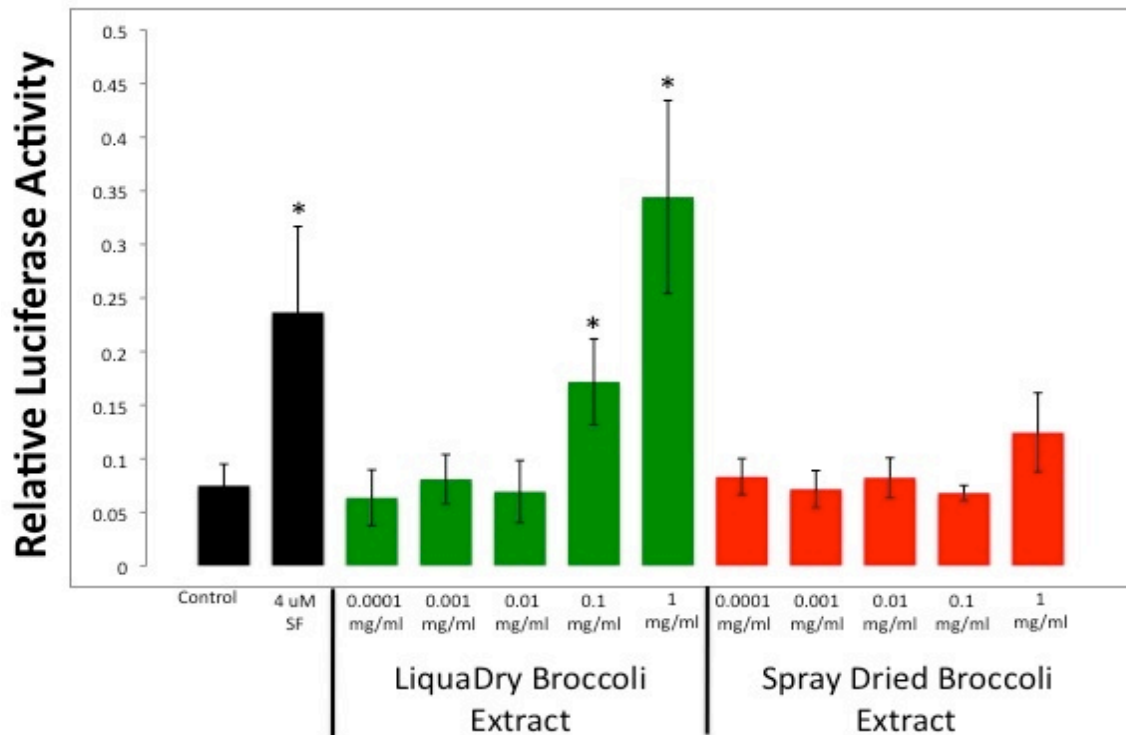


Figure 4. Effect of thermal processing on broccoli extract bioactivity. Columns are means (n=8) ± standard deviation. Columns marked with an asterisk are significantly different from control (P < 0.05).

Work yet to complete

1. Comparison of LiqueDry extract to broccoli juice
2. Sulforaphane content of spray dried and liquaDry extracts

Literature Cited

[1] Michaud, D. S., Spiegelman, D., Clinton, S. K., Rimm, E. B., *et al.*, Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *J Natl Cancer Inst* 1999, 91, 605-613.

[2] Terry, P., Wolk, A., Persson, I., Magnusson, C., Brassica vegetables and breast cancer risk. *JAMA* 2001, 285, 2975-2977.

[3] Kolonel, L. N., Hankin, J. H., Whittemore, A. S., Wu, A. H., *et al.*, Vegetables, fruits, legumes and prostate cancer: a multiethnic case-control study. *Cancer Epidemiol Biomarkers Prev* 2000, 9, 795-804.

[4] Jain, M. G., Hislop, G. T., Howe, G. R., Ghadirian, P., Plant foods, antioxidants, and prostate cancer risk: findings from case-control studies in Canada. *Nutr Cancer* 1999, 34, 173-184.

[5] Finley, J. W., The antioxidant responsive element (ARE) may explain the protective effects of cruciferous vegetables on cancer. *Nutr Rev* 2003, 61, 250-254.

- [6] Talalay, P., Fahey, J. W., Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J Nutr* 2001, *131*, 3027S-3033S.
- [7] Van Eylen, D., Oey, I., Hendrickx, M., Van Loey, A., Kinetics of the stability of broccoli (*Brassica oleracea* Cv. Italica) myrosinase and isothiocyanates in broccoli juice during pressure/temperature treatments. *J Agric Food Chem* 2007, *55*, 2163-2170.
- [8] Jin, Y., Wang, M., Rosen, R. T., Ho, C. T., Thermal degradation of sulforaphane in aqueous solution. *J Agric Food Chem* 1999, *47*, 3121-3123.
- [9] Hintze, K. J., Wald, K., Finley, J. W., Phytochemicals in broccoli transcriptionally induce thioredoxin reductase. *J Agric Food Chem* 2005, *53*, 5535-5540.
- [10] Hintze, K. J., Theil, E. C., DNA and mRNA elements with complementary responses to hemin, antioxidant inducers, and iron control ferritin-L expression. *Proc Natl Acad Sci U S A* 2005, *102*, 15048-15052.
- [11] Finley, J. W., Sigrid-Keck, A., Robbins, R. J., Hintze, K. J., Selenium enrichment of broccoli: interactions between selenium and secondary plant compounds. *J Nutr* 2005, *135*, 1236-1238.
- [12] Hintze, K. J., Wald, K. A., Zeng, H., Jeffery, E. H., Finley, J. W., Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. *J Nutr* 2003, *133*, 2721-2727.
- [13] Hintze, K. J., Keck, A. S., Finley, J. W., Jeffery, E. H., Induction of hepatic thioredoxin reductase activity by sulforaphane, both in Hepa1c1c7 cells and in male Fisher 344 rats. *J Nutr Biochem* 2003, *14*, 173-179.