

2004 MAFMA Final Report

Project Title: Health Benefits of Frozen Broccoli
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1. Objective Summary

To investigate the changes in content of Glucoraphanin and its hydrolysis products sulforaphane and nitrile, as well as total phenolic content of fresh broccoli when it is subjected to blanching, freezing, thawing, and cooking, and how to use this information to optimize processing of frozen broccoli florets for content of these bioactive components. If phenolics and sulforaphane can be enhanced (and nitrile diminished) then broccoli will provide greater health benefits. We have published that there is a cofactor to myrosinase, the enzyme that hydrolyzes glucosinolates when broccoli is chopped, that directs the unstable intermediate hydrolysis product to become nitrile (which has no known health benefit) in place of sulforaphane (known as the component responsible for broccoli's anti-cancer action). Work with the purified cofactor suggests that it is far more heat sensitive than myrosinase. If this is the case, then processing of frozen broccoli may be optimized for sulforaphane production. Yet this would only be of use, if sufficient heating could be utilized to destroy peroxidase. As a secondary concern, antioxidants have been reported to be labile to processing in some plant foods. We therefore chose to monitor broccoli antioxidant response to steam-blanching, freezing and thawing.

2. Objective Accomplishments

Preliminary work: Fresh broccoli was steamed, microwaved, or boiled for various times and the fractional formation of sulforaphane, compared to the inactive nitrile, compared upon chopping the broccoli and allowing hydrolysis to occur. We found that boiling and microwaving for as little as one minute (followed by rapid cooling in cold water) caused a rapid loss not only in nitrile formation, but also in sulforaphane formation, suggesting that the hydrolyzing enzyme myrosinase had been destroyed by the heat, see Figure 1. By contrast, steaming produced a slower process of heat-denaturation, such that sulforaphane production was substantially enhanced by steaming broccoli for 1 – 3 minutes, compared to the amount of sulforaphane formed when fresh broccoli was chopped.

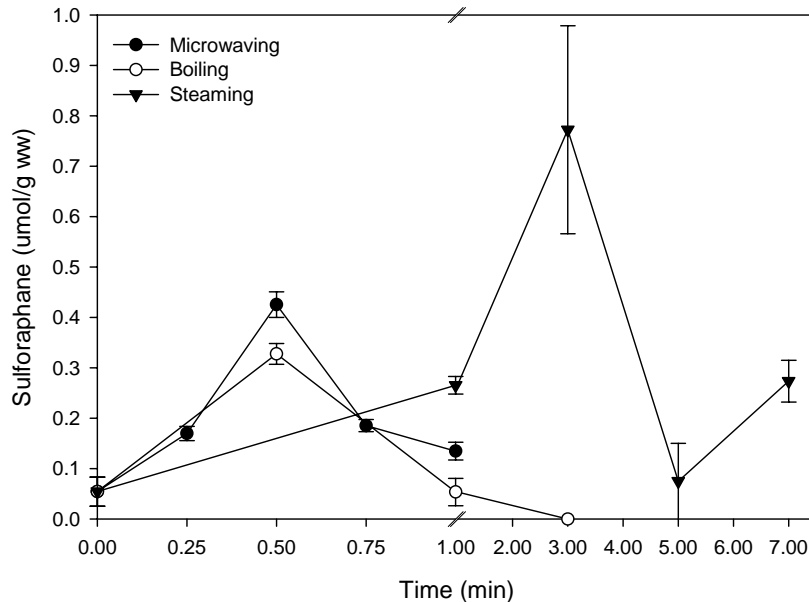


Figure 1. Preliminary work Data: Effect of heating by microwaving, boiling, and steaming on sulforaphane levels ($\mu\text{mol/g ww}$) (Pinnacle). Data are expressed as mean \pm SE (n=3).

Part 1: Steam Blanching

The specific objective of part 1 was to determine the optimal steam blanching time for sulforaphane production. We found that steam blanching broccoli florets for 2 min produced 3.8-fold higher sulforaphane content compared to that in raw broccoli: 0.71 compared to 0.19 $\mu\text{mol/g}$ fresh broccoli. In the same samples, the non-bioactive sulforaphane nitrile decreased by 21-fold from 0.26 down to 0.01 $\mu\text{mol/g}$ fresh broccoli, see Figure 2.

This short steam blanching time decreased the phenolic content in the broccoli floret by about 20% compared to raw broccoli, see Figure 3, but completely inhibited the off-flavor producing peroxidase activity that is active in raw broccoli (Figure 4). Repeating the phenolic content in raw and steamed (first two bars in Figure 5), we saw $\sim 10\%$ loss, which was not significant. Steaming for 2 min did not alter content of any single glucosinolate or the total glucosinolate level in the broccoli. The glucoraphanin concentration was 11.8 ± 2.2 $\mu\text{mol/g}$ dry weight in raw broccoli and 10.8 ± 0.6 $\mu\text{mol/g}$ dry weight in 2 min steamed broccoli.

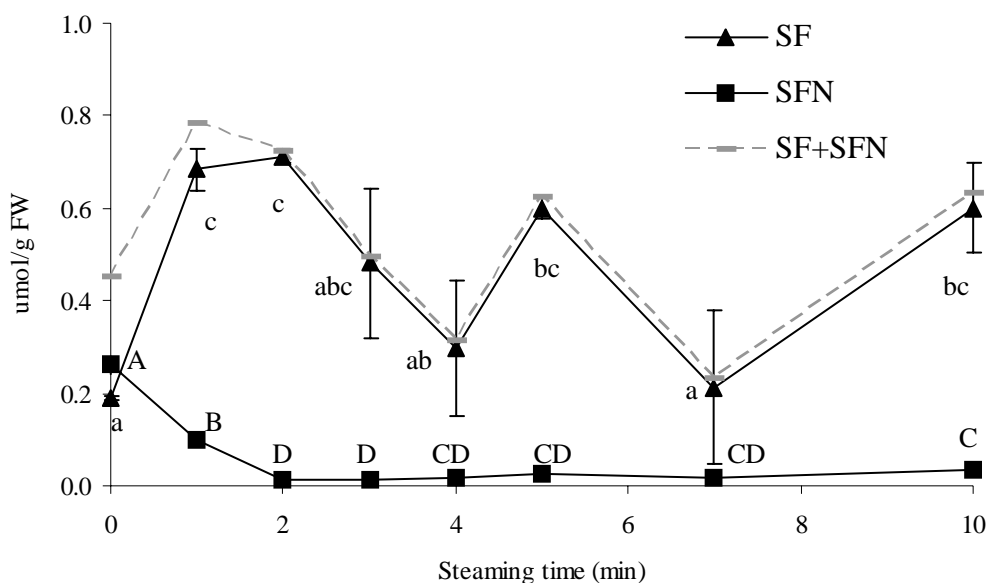


Figure 2. Sulforaphane and sulforaphane nitrile levels in hydrolyzed steam-blanch broccoli. Market mature broccoli (var. Marathon) was harvested and representative samples of 100g were steamed blanch in a Flavor Scenter Handy Steamer (HS800, Black and Decker) for 0, 1, 2, 3, 4, 5, 7, and 10 min. After cooling on ice for 5 minutes and blotting dry, samples were chopped. Triplicate 10g samples were homogenized using a tissue-mizer in 10 ml dH₂O and hydrolyzed for 8 hrs before sulforaphane and sulforaphane nitrile levels were measured by GC using our published method (Matusheski et al. 2001). Data shown are mean ± SE (n=3). Different letters indicate values that are significantly different (ANOVA, LSD, p<0.05).

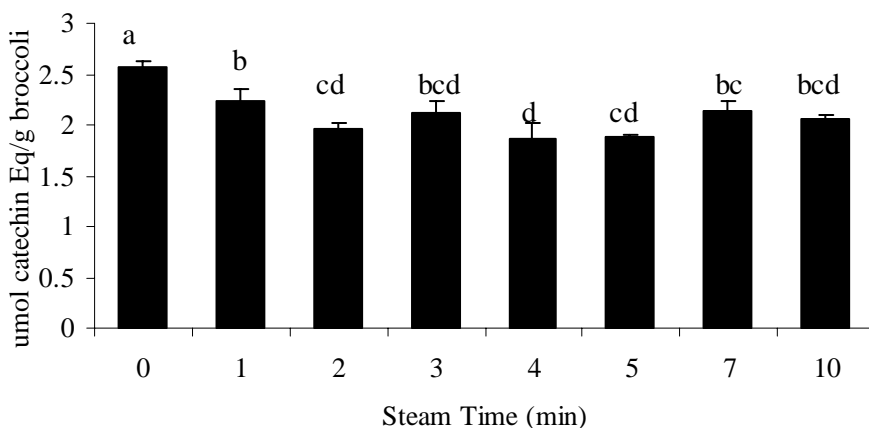


Figure 3. Phenolic content of hydrolyzed steam-blanch broccoli. Phenolics were analyzed in triplicate on hydrolyzed steam-blanch broccoli samples using the Folin-Ciocalteu assay (Swain 1959). Results were expressed in umol catechin equivalents per gram broccoli. Data shown are mean ± S.E. (n=3). Different letters indicate significant differences (ANOVA, LSD, p<0.05).

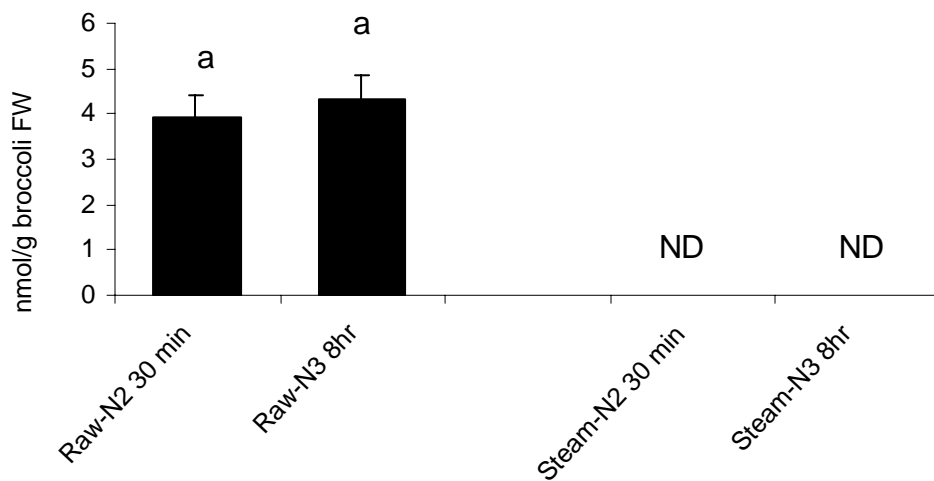


Figure 4. Peroxidase activity of broccoli, raw or 2 minutes steam-blanching, then hydrolyzed for 30 minutes or 8 hours. A qualitative assay will be used to determine peroxidase activity (Miller 1998). Data shown are mean \pm S.E. (n=3). Since 2 minutes of steam-blanching inactivated the enzyme, we did not analyze other samples that were heated for longer.

Part 2: Freezing, Thawing, Cooking

The specific objective of part 2 was to determine the effect of freezing, thawing and cooking of the steam-blanching broccoli florets produced in part 1.

Phenolics: There was no difference in phenolic content of broccoli florets that were steam-blanching for 2 min, snap frozen and analyzed without thawing, compared to broccoli florets that, after the steam-blanching, were either a) frozen and then thawed quickly in cold water, b) fridge thawed or c) thawed in the microwave oven for 30 sec

Compared to raw broccoli, or broccoli florets that were steam-blanching and snap frozen in liquid nitrogen, phenolic content was significantly decreased in broccoli that, following steam-blanching, then freezing to -20°C , were either a) thawed in the microwave oven for 1 or 3 min, b) thawed by steam cooking for 3, 5 or 7 min or c) thawed by the thaw cycle in the microwave oven, see Figure 5.

Glucosinolates: Although steam blanching and freezing caused no loss in glucosinolate content, glucosinolates were lost by all thawing methods.

Sulforaphane: Fridge thawing provided greater sulforaphane and less nitrile formation than a quick defrost in water or use of the microwave defrost cycle. When frozen samples were microwave cooked or steam cooked, sulforaphane was lost after 1 and 3 minutes, respectively.

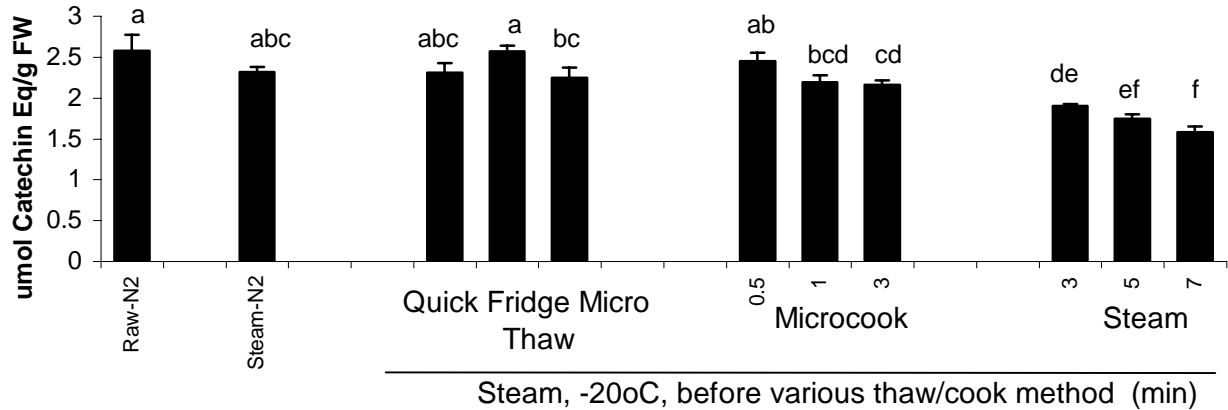


Figure 5. Phenolic content of broccoli hydrolyzed for 8 hours. Phenolics were analyzed from three independent preparations and from duplicate samples from each preparation, using the Folin-Ciocalteu assay (Swain 1959). Results expressed in umol catechin equivalents per gram broccoli. Data shown are mean \pm S.E. (n=3). Different letters indicate significant differences (ANOVA, LSD, $p < 0.05$).

3. Practical impacts of research efforts.

a. Short Term Impacts

Broccoli florets that are steam-blanching for a short time (2 min) before freezing may increase the content of bioactive antioxidants and sulforaphane, hence provide greater disease preventive power, but still destroy the peroxidase that is associated with both off-flavors and shortened shelf life.

b. Long Term Impacts

These findings may lead to changes in the processing procedure of the industry so that short-term steam blanching will be standard before freezing. This would provide the general public with broccoli with higher health promoting ability. Because conditions vary considerable from the laboratory to the processing plant, it would be necessary to determine the optimum time for blanching, able to destroy peroxidase and enhance sulforaphane levels. However, these studies suggest that such a time could be found.