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PHYTOCHEMICALS IN SPANISH BLACK RADISH INDUCE DETOXIFICATION ENZYMES

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Abstract

To better understand the physiological effect of Spanish black radish (SBR), a rich source of glucosinolates and their metabolites, the prevalence and effectiveness of these constituents contained in SBR were examined. SBR glucosinolates were putatively identified and the effects of SBR extracts on detoxification enzyme activity were determined. HPLC analysis of lyophilized whole, halved, quartered, and sliced SBR confirmed a high abundance of the glucosinolate 4-methylthiobut-3-enyl (4-MTB-3-E), whereas lyophilized ground or high temperature dried SBR demonstrated very little 4-MTB-3-E. SBR aqueous extracts induced three detoxification enzymes in the Hepa-1c1c7 immortalized mouse hepatoma cell line: glutathione S-transferase, quinone reductase (QR), and thioredoxin reductase. The data demonstrate that the aqueous extract of SBR is a more potent inducer of QR activity than the aqueous extract of other crucifers (kale and Brussels sprouts). Moreover, following induction by SBR QR activity remained elevated for seven days even after SBR extract had been removed from the cells. However, certain active biological constituents of SBR remain unknown. It is now recognized that SBR contains high levels of one major glucosinolate, multiple breakdown products of this glucosinolate confirmed by GC-MS, and the capacity to induce detoxification enzymes in liver cells. *Work supported by Standard Process Inc.*

Introduction

A diet rich in fruits and vegetables and, in particular, cruciferous vegetables has been linked to a decrease in risk for many chronic diseases, including cancer.¹ The chemopreventative properties of cruciferous vegetables have been linked to their production of a class of phytochemicals referred to as glucosinolates. Glucosinolates are present in whole crucifer plants in high concentration. Upon tissue disruption (such as grinding), the glucosinolates are metabolized, by an enzyme also contained within the crucifer called myrosinase, into numerous breakdown products, such as nitriles and isothiocyanates, that are believed to be the active phytochemical compounds. These glucosinolate breakdown products have been shown in both in vivo and in vitro models to be able to induce detoxification enzymes²⁻⁷ and also to be anticarcinogenic in some models.⁸⁻¹⁰ Thus, it has been suggested that induction of detoxification enzymes is at least one of the mechanisms through which cruciferous vegetable consumption, or the consumption of the glucosinolate breakdown products, reduces cancer risk.¹¹⁻¹³



Spanish black radish, *Raphanus sativus L. var niger*, is a cruciferous vegetable in the same family as broccoli, kale, Brussels sprouts, and spinach. Spanish black radishes range from the size of a golf ball to the size of a softball.

Spanish black radish, also known as *Raphanus sativus L. var niger*, has been shown in general to have high total glucosinolate content in comparison with other cruciferous vegetables.¹⁴ Interestingly, a unique glucosinolate purified and identified over a decade ago, 4-methylthio-3-butenyl or glucoraphasatin,¹⁵⁻¹⁶ constitutes a vast majority of the glucosinolates present in radishes.¹⁴ While there are studies demonstrating the antioxidant^{15,17,18} and antimutagenicity¹⁹ of radishes and phytochemicals derived from radishes, there have been no published reports of radishes increasing detoxification enzyme activity. The high glucosinolate concentration of radish suggests the possibility that Spanish black radish may be a potent inducer of detoxification enzymes when individuals are exposed through the diet. The presence of a unique glucosinolate in radishes presents the possibility of isolating and studying novel compounds that may have activity similar to the known anti-carcinogenic compound sulforaphane that is found in other members of the crucifer family.

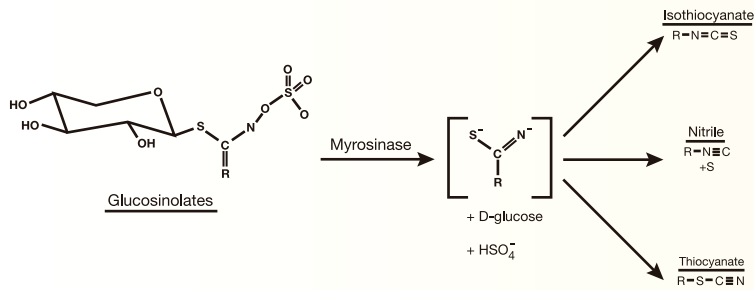


Figure 1. Myrosinase metabolism of glucosinolates.

Upon grinding of tissue, myrosinase enzyme within the plant metabolizes glucosinolates into various breakdown products that have been implicated as the biologically active components of cruciferous vegetables.

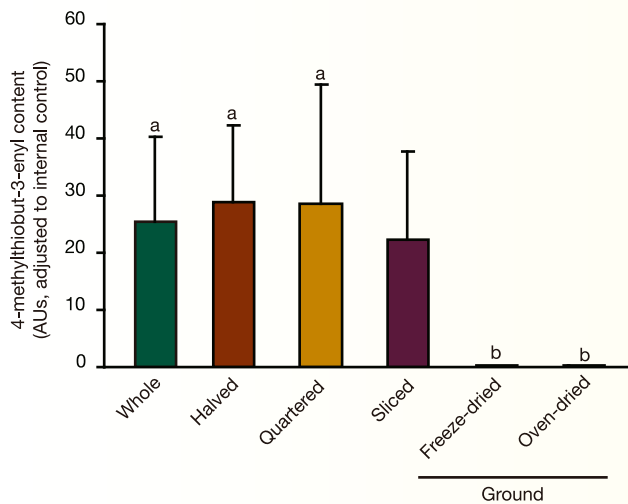


Figure 2. Grinding radishes reduced glucoraphasatin concentration.

As expected, grinding radishes reduced glucosinolate content, most likely through metabolism of glucosinolates by endogenous myrosinase.

Typically, 4-methylthiobut-3-enyl (glucoraphasatin) made up approximately 80% of all peaks on the chromatogram from radishes.

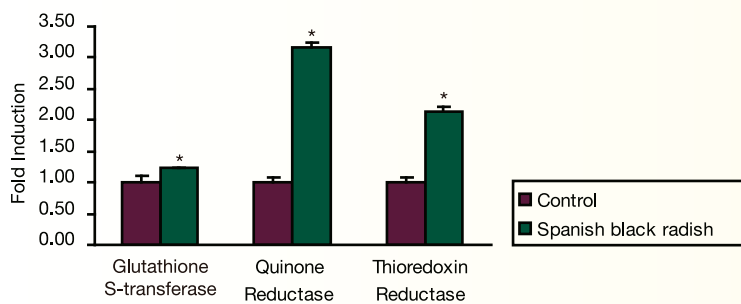


Figure 3. The aqueous extract from Spanish black radish increased activity of three detoxification enzymes in Hepa-1c1c7 cells.

Hepa-1c1c7 cells were treated for 2 days with the aqueous extract from ~7.3 mg of Spanish black radish (4 mg of dried extract).

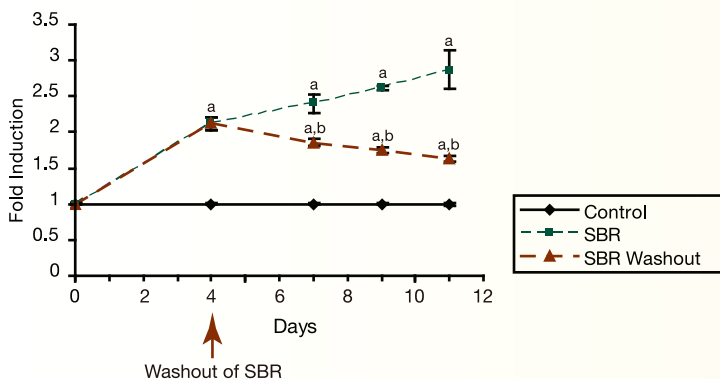


Figure 4. Cells treated with Spanish black radish (SBR) retain elevated Quinone Reductase activity up to a week after extract is removed from the cells.

HepG2 cells were treated with 4 mg of aqueous SBR extract for four days before being washed out of one set of cells (SBR Washout). Another set of cells received 4 mg of aqueous SBR extract continuously through 11 days (SBR).

a = significantly different from control
b = significantly different from continuous SBR treatment

Quinone Reductase activity in cells continuously treated with aqueous SBR extract increased through 11 days of treatment.

Removing SBR extract from cells significantly decreased Quinone Reductase activity, but activity of these cells still remained elevated over control-treated cells.

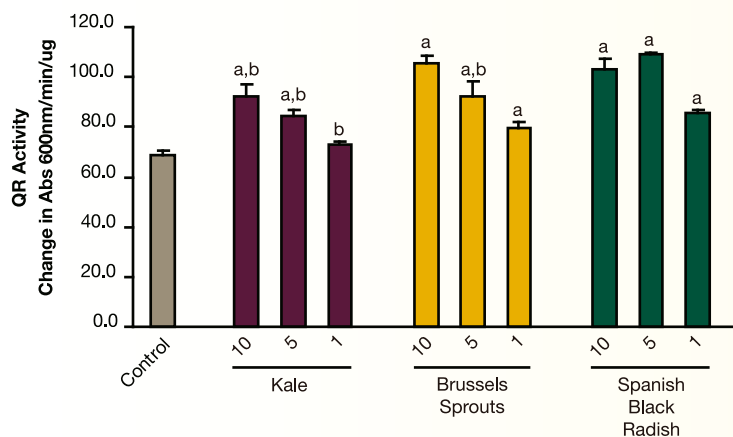


Figure 5. The aqueous extract from Spanish black radish is at least as potent as two other cruciferous vegetables at inducing Quinone Reductase activity.

HepG2 cells were treated for 2 days with the amount of aqueous extract of the three crucifers equivalent to 10, 5 and 1 mg of dry starting material.

a = significantly different from control
b = significantly different from Spanish black radish at the same concentration

The aqueous extract from all three crucifers induced Quinone Reductase activity in a dose-dependent manner.

Aqueous extract from 5 mg of Spanish black radish was more potent at inducing Quinone Reductase activity than 5 mg of kale or Brussels sprouts.

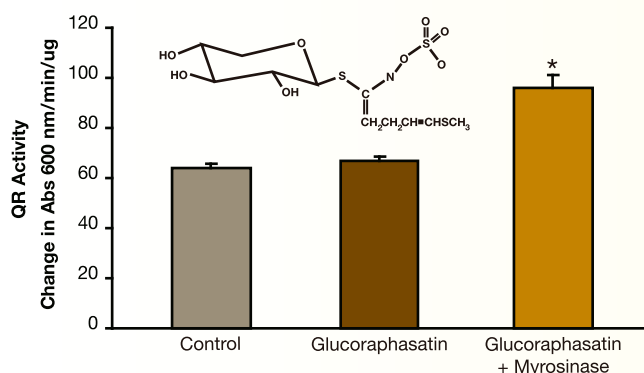


Figure 6. Glucoraphasatin induces Quinone Reductase activity, only after metabolism by myrosinase.

HepG2 cells were treated with 2.4 mg of glucoraphasatin after overnight incubation at room temperature with either water alone (center bar) or with purified myrosinase enzyme (right bar).

The myrosinase metabolites of glucoraphasatin, but not the glucosinolate parent compound itself, was able to significantly induce Quinone Reductase activity.

Conclusions

The aqueous extract from Spanish black radish activates Phase II enzyme activity in an immortalized hepatocyte cell model. Spanish black radishes are an intriguing cruciferous vegetable to study because:

- Spanish black radishes have a high concentration of glucosinolates.
- Glucoraphasatin, a glucosinolate unique to the radish family, makes up approximately 80% of all the glucosinolates in the radish.

- Metabolites of glucoraphasatin produced by myrosinase induce Quinone Reductase.
- Aqueous extracts from Spanish black radish are as potent or more potent than other crucifer extracts at inducing detoxification enzymes.
- Few published reports have examined the biological activity of radishes, as opposed to a growing amount of literature focusing on broccoli and its phytochemicals.

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