



An Investigative Study of Reactions Involving Glucosinolates and Isothiocyanates

Alzea Chrisel H. Alea¹, Diane Elaine T. Co² and Marissa G Noel^{3*}

^{1,2,3}Chemistry Department, De La Salle University, 2401 Taft Ave., Manila

*Corresponding Author: marissa.noel@dlsu.edu.ph

Abstract: Glucosinolates (GSL) are thioglucosides normally found in Brassica vegetables and most are known to have health promoting properties. Some of their degradation products such as isothiocyanates have been shown to exhibit chemopreventive action.

The availability, accessibility and properties of glucosinolates and their hydrolysis products may however be affected if they react with other cellular components such as metal ions. In this study reaction mixtures consisting of a glucosinolate (sinigrin) and a metal ion (Fe^{3+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Mg^{2+} , and Ni^{2+}) and an isothiocyanate (allyl isothiocyanate) with a metal ion were analyzed by UV-Vis Spectrophotometry to determine possible interactions under physiological conditions. Both sinigrin and allyl isothiocyanate exhibited apparent interactions with aqueous solutions of FeCl_3 , $\text{Cu}(\text{NO}_3)_2$ and $\text{Zn}(\text{NO}_3)_2$. Based on the difference spectra generated, no significant interactions were found between the standards and Fe^{2+} , Mg^{2+} and Ni^{2+} salts. These findings show that glucosinolates and isothiocyanates have promising anti-Fenton activity. Further investigation on the interactions with Fe^{3+} was made by analysis of the reaction mixtures by high performance liquid chromatography (HPLC) using a refractive index detector and by liquid chromatography – mass spectrometry (LC-MS). Results from HPLC analysis revealed no significant covalent interactions between Fe^{3+} and either sinigrin or allyl isothiocyanate. However, LC-MS analysis showed a prominent ion at m/z 413 with the formula $[\text{C}_{10}\text{H}_{16}\text{KO}_9\text{S}_2\text{Fe}]^+$, the probable product formed between Fe^{3+} and sinigrin. Analysis of the Fe^{3+} - allyl isothiocyanate reaction mixture also indicated the formation of a possible product with the formula $[(\text{C}_4\text{H}_5\text{NS})_3\text{Fe} + \text{H}]^+$ at m/z 353.

KEYWORDS: glucosinolate; sinigrin; allyl isothiocyanate; LC-MS (liquid chromatography – mass spectrometry)

1. INTRODUCTION

Glucosinolates are β -thioglucoside *N*-hydroxysulfates often associated with cruciferous vegetables. These compounds are biologically inactive but are hydrolysed by the enzyme myrosinase yielding products that can have medicinal applications. The hydrolysis products include isothiocyanates some of which have been shown to exhibit chemopreventive properties. However, the availability, accessibility and properties of glucosinolates and their degradation products may be affected if they react with other cellular components.

This research sought to investigate possible reactions involving glucosinolates and isothiocyanates which have the potential to enhance or reduce their biological activities. Interactions with metal ions which play important roles in human metabolism or which could affect normal physiological processes were studied. Reaction mixtures consisting of standard solutions of glucosinolate (sinigrin) or isothiocyanate (allyl isothiocyanate) and one of the following metals: Fe^{3+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Mg^{2+} , Ni^{2+} , Pb^{2+} in buffered solution were analyzed by UV-Vis spectrophotometry. The interactions of Fe^{3+} with sinigrin and allyl isothiocyanate were further studied by high performance liquid chromatography (HPLC) and liquid chromatography – mass spectrometry (LC-MS).

2. METHODOLOGY

2.1 Materials, Chemicals and Reagents

Sinigrin (allyl glucosinolate) used as the standard glucosinolate, allyl isothiocyanate (standard isothiocyanate), and the hydrolytic enzyme myrosinase (E.C. No. 3.2.1.1.147) were all purchased from Sigma-Aldrich. The reagents used as sources of metal ions: (Cu^{2+} , Fe^{2+} , Fe^{3+} , Pb^{2+} , Mg^{2+} , Ni^{2+} , Zn^{2+}) were AR grade copper nitrate, ferrous ammonium sulfate, ferric chloride, lead nitrate, nickel sulfate, magnesium chloride and zinc nitrate respectively. These were prepared as solutions dissolved in deionized water. HPLC grade acetonitrile was used for HPLC and LCMS analysis. Methanol and DCM (dichloromethane) used as solvents were analytical grade.

2.2 Reactions with Metals

To determine possible interactions between metal ions and glucosinolates as well as metal ions and isothiocyanates, UV-Vis absorption spectra of standard sinigrin (5 mM), standard isothiocyanate (5 mM), solutions of metal ions (5 mM) and their respective mixtures were taken.

Standard: A measured volume (0.1 mL) of standard and 2.0 mL of phosphate buffer (pH 6.5) were placed in a quartz cuvette for absorbance reading at a wavelength range of 200 - 500 nm with 10 nm intervals. Water was used as blank.

Metal ions: Varying amounts of 5 mM solutions of metal ions (i.e. Cu^{2+} , Fe^{2+} , Fe^{3+} , Pb^{2+} , Mg^{2+} , Ni^{2+} , Zn^{2+}): were added to 2.0 mL of phosphate buffer (pH 6.5) in a quartz cuvette. Wavelength scans (200 – 500 nm) of these solutions were taken. Water was used as a blank.

Interaction mixtures: A constant amount of standard (sinigrin or allyl isothiocyanate) was mixed with varying amounts of metal solution (interaction mixtures) to determine their interaction. In a quartz cuvette, 0.1 mL of standard, 2.0 mL of phosphate buffer (pH 6.5) and varying amounts of metal solution were mixed and absorbances of these mixtures were read at 200-500 nm with 10 nm intervals..

2.3 HPLC and LC-MS Analysis

Reaction mixtures prepared from 5 mM FeCl_3 and standard sinigrin / allyl isothiocyanate were analyzed by high performance liquid chromatography (HPLC) using a refractive index detector. Separation was done using a 250 x 4.6 mm C18, 5 μm reversed phase HPLC column. Elution was carried out using an isocratic solvent system consisting of 85 % water and 15% acetonitrile at a flow rate of 1.0 mL/min.

LC-MS analysis of the reaction mixtures was done on a DIONEX Ultimate 3000 HPLC equipped with a Bruker MicroTOF QII MS.

3. RESULTS AND DISCUSSION

3.1 UV-Vis Analysis

To investigate the presence of glucosinolate (or isothiocyanate)-metal interactions,

a wavelength scan of the standard (sinigrin/ allyl isothiocyanate) was first made. Varying concentrations of the metal ion ($\text{Cu}^{2+}/ \text{Fe}^{2+}/ \text{Fe}^{3+}/ \text{Mg}^{2+}/ \text{Ni}^{2+}/ \text{Zn}^{2+}/ \text{Pb}^{2+}$) in solution were then analyzed and their absorbances at different wavelengths determined. Finally, the absorption spectra of the interaction mixtures (standard and varying concentrations of the metal ion) were obtained.

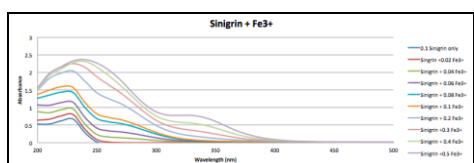


Fig. 1 Absorption spectra of interaction mixtures (sinigrin + varying concentrations of $\text{FeCl}_3(\text{aq})$)

The λ_{max} of sinigrin was found to be at 230 nm. The absorption spectrum found for FeCl_3 was consistent with earlier studies indicating the presence of Fe^{3+} and FeCl_2^+ at $\lambda_{\text{max}} = 240$ nm and 339 nm respectively (Stefansson, *et al.*, 2008).

To determine the presence or absence of interactions between sinigrin and Fe^{3+} , a mathematical analysis was performed using the spectra shown in Fig 1. This was based on the presumption that when no interactions exist, the two absorbing species would retain their individual spectra, in which case the total absorption of light at any given wavelength would just be the sum of the absorbances of the two separate species measured under the same conditions.

A simple formula was used to generate subtracted spectra for the interaction studies done using a fixed concentration of standard glucosinolate (or isothiocyanate) and varying amounts of metal ion. A flat difference spectrum (absorbance = zero) indicated simple addition of the absorbances of the components of the mixture, which meant that no interaction existed between them. On the other hand, a notable difference (whether in the positive or negative direction) between the mathematical sum of absorbances of the species and that of the mixture would be evidence of interactions between the components of the mixture.

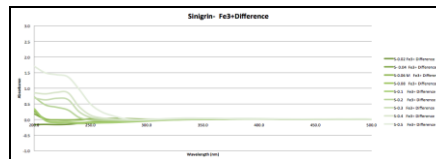


Fig. 2 Subtracted spectra for sinigrin and $\text{FeCl}_3(\text{aq})$

The results illustrated in Figure 2 show evident positive differences upon calculation. This signifies very probable existence of interaction between sinigrin and Fe^{3+} . The positive difference indicates that the sum of the individual absorbances of sinigrin and iron (III) was higher than that of the mixture. The absorbing species in Figure 1 could be a product of the interaction. This is supported by the observed shifts in band position and change in band shape as well as the increase in absorbance at 220 nm shown in Figure 3.

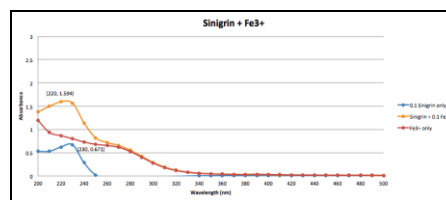


Fig. 3. Absorption spectra of sinigrin, Fe^{3+} only and interaction mixture (sinigrin + Fe^{3+})

Oxidation reactions induced by transition metals such as Fe and Cu produce free hydroxyl radicals, which may cause death or damage to cellular components in the body. These reactions known as Fenton reactions explain why free iron (either as Fe^{3+} or Fe^{2+}) is considered toxic to the body even at low concentrations (10^{-8} M). The presence of metal chelators capable of reacting with free iron forming less reactive compounds would reduce these potentially harmful reactions (Guo, *et al.*, 2007, Wei and Guo, 2007).

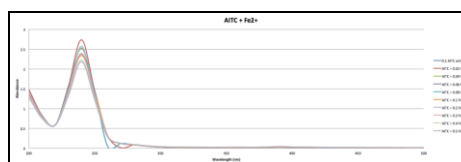


Fig. 4 Interaction mixtures (allyl isothiocyanate + varying concentrations of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2(\text{aq})$)

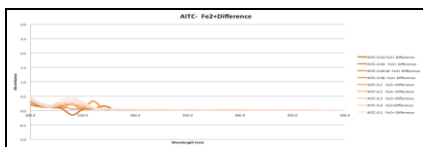


Fig 5. Subtracted spectra for allyl isothiocyanate and $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2(\text{aq})$

Data in Figures 4 and 5 indicate both positive and negative differences, which could suggest possible interactions between allyl isothiocyanate and $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2(\text{aq})$. However, the differences between the sums of the spectra of the individual species and the mixture were relatively small (O.D. <0.5). Based on the data available no definite conclusion regarding the interactions between allyl isothiocyanate and $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2(\text{aq})$ can be given at present.

Table 1. Summary of results from UV-Vis Analysis

| | Sinigrin | Allyl Isothiocyanate |
|--|----------|-------------------------|
| $\text{FeCl}_3(\text{aq})$ | (+) | (+) |
| $\text{Cu}(\text{NO}_3)_2(\text{aq})$ | (+) | (+) |
| $\text{Zn}(\text{NO}_3)_2(\text{aq})$ | (+) | (+) |
| $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2(\text{aq})$ | (-) | |
| $\text{MgCl}_2(\text{aq})$ | (-) | |
| $\text{NiSO}_4(\text{aq})$ | (-) | |
| $\text{Pb}(\text{NO}_3)_2(\text{aq})$ | (+) | |
| $\text{ZnCl}_2(\text{aq})$ | | |

(+) very probable interactions observed
 (-) no significant interactions

Sinigrin was found to have significant interactions with $\text{FeCl}_3(\text{aq})$, $\text{Cu}(\text{NO}_3)_2(\text{aq})$, $\text{Zn}(\text{NO}_3)_2(\text{aq})$ and $\text{Pb}(\text{NO}_3)_2(\text{aq})$. Its hydrolysis product, allyl isothiocyanate showed significant interactions with $\text{FeCl}_3(\text{aq})$, $\text{Cu}(\text{NO}_3)_2(\text{aq})$ and $\text{Zn}(\text{NO}_3)_2(\text{aq})$. The results of the spectrophotometric analyses are summarized in Table 1. (Note: No entries were made for results where no definite conclusions could be drawn.)

3.2 HPLC Analysis

Further investigation was made to determine the nature of interactions existing between glucosinolates / isothiocyanates and Fe^{3+} ions. Since UV-Vis spectrophotometric analysis

showed very promising interactions, HPLC analysis of the reaction mixture was done to verify the results.

Three components: $\text{FeCl}_3(\text{aq})$, sinigrin and water are identifiable from the chromatogram in Figure 6. The addition of more sinigrin only increased its peak area. This suggests that no significant covalent interactions exist between sinigrin and Fe^{3+} because an RI detector would have detected a compound probably with a different retention time if such interactions existed. There is however, the possibility of ionic interactions existing between sinigrin and Fe^{3+} . The chromatogram in Figure 7 also indicates that no covalent interactions were observable between Fe^{3+} and allyl isothiocyanate.

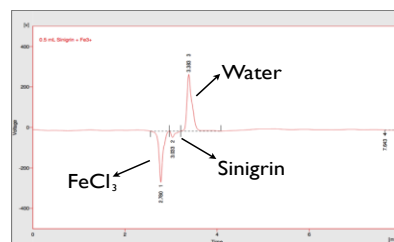


Fig. 6 HPLC chromatograms of Fe^{3+} + (a) 0.5 mL sinigrin; (b) 1.0 mL sinigrin

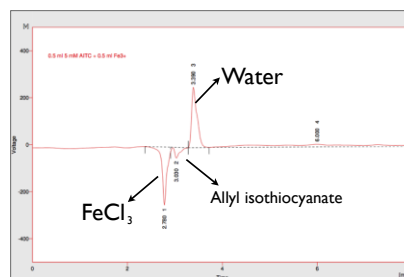


Fig. 7 HPLC chromatogram of allyl isothiocyanate + $\text{FeCl}_3(\text{aq})$

3.3 LC-MS Analysis

The base peak chromatogram showed three distinct peaks: one had a mass spectrum of showing a pattern which could represent ferric chloride, the mass spectrum of second compound gave an intense ion fragment at m/z 413. This fragment was not evident in the mass spectra of

$\text{FeCl}_3(\text{aq})$ and sinigrin. This could be that of a product formed between Fe^{3+} and sinigrin with a formula of $[\text{C}_{10}\text{H}_{15}\text{FeNO}_9\text{S}_2]^+$. We propose a possible structure where Fe is bound to the glucose moiety.

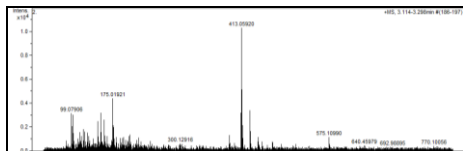
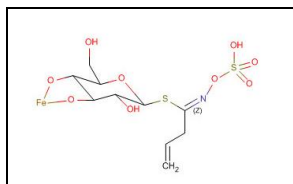
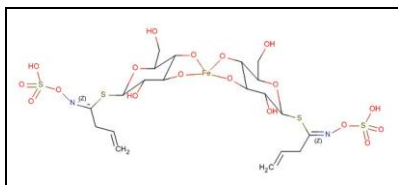


Fig. 8. Mass spectrum of a BPC peak



Proposed Structure of Fe-Sinigrin Product

A less intense ion at m/z 770 may be attributed to the formula of $[\text{C}_{20}\text{H}_{30}\text{FeN}_2\text{O}_{18}\text{S}_4]^+$. A possible structure of the product is proposed which involves two sinigrin molecules bound to Fe.



Proposed Structure of product at m/z 770

Analysis of the Fe - allyl isothiocyanate reaction mixture also showed a possible product formed with m/z 353 which could be that of a protonated molecular ion with the formula $[(\text{C}_4\text{H}_5\text{NS})_3\text{Fe} + \text{H}]^+$.

4. CONCLUSIONS

Interactions of glucosinolates and isothiocyanates with metal ions were studied. Spectrophotometric analysis showed that both sinigrin and its hydrolytic product allyl isothiocyanate reacted with Fe^{3+} , Cu^{2+} and Zn^{2+} . These findings pose relevant biological implications, one of which is the potential anti-Fenton activity of these compounds.

LC-MS analysis of the reaction mixtures with Fe^{3+} yielded fragment ions which could be those of $[\text{C}_{10}\text{H}_{15}\text{FeNO}_9\text{S}_2]^+$ and $[(\text{C}_4\text{H}_5\text{NS})_3\text{Fe} + \text{H}]^+$ representing products formed with sinigrin and allyl isothiocyanate respectively.

5. ACKNOWLEDGMENTS

The authors wish to acknowledge the help and support extended by the faculty of the Chemistry Department.

6. REFERENCES

- Guo, M., Perez, C., Wei, Y., Rapoza, E., Su, G., Bou-Abdallah, F. & Chasteen, N.D. (2007). Iron-binding properties of plant phenolics and cranberry's bio-effects. *Dalton Trans*, 21 (43), 4951 – 4961.
- Wei, Y. & Guo, M. (2007). Hydrogen peroxide triggered prochelator activation, subsequent metal chelation, and attenuation of the Fenton reaction. *Angew. Chem Int Ed Engl*, 46 (25), 4722 – 4725.
- Stefansson, A., Lemke, K. & Seward, T. (2008). Iron (III) complexation in hydrothermal solutions – an experimental and theoretical study, In *Proceedings of ICPWS XV* (15th Internat. Conference on the Properties of Water and Steam), Berlin.