

# Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables

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## Abstract

Epidemiological studies have shown that consumption of *Brassica* vegetables decrease the risk of cancer. These associations are linked to dietary intake of glucosinolates and their metabolism to cancer preventive isothiocyanates. Bioavailability of glucosinolates and related isothiocyanates are influenced by storage and culinary processing of *Brassica* vegetables. In this work, the content of the 7 major glucosinolates in broccoli, Brussels sprouts, cauliflower and green cabbage and their stability under different storage and cooking conditions is examined. Glucosinolates and isothiocyanates were quantified by liquid chromatography with tandem mass spectrometric detection (LC–MS/MS). Isothiocyanates were detected with high sensitivity as the corresponding thiourea derivatives. Storage at ambient temperature and in a domestic refrigerator showed no significant difference and a minor loss (9–26%) of glucosinolate levels over 7 days. Vegetables shredded finely showed a marked decline of glucosinolate level with post-shredding dwell time – up to 75% over 6 h. Glucosinolate losses were detected partly as isothiocyanates. Cooking by steaming, microwaving and stir-fry did not produce significant loss of glucosinolates whereas boiling showed significant losses by leaching into cooking water. Most of the loss of the glucosinolates (~90%) was detected in the cooking water. Increased bioavailability of dietary isothiocyanates may be achieved by avoiding boiling of vegetables.

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**Keywords:** *Brassica* vegetables; Glucosinolates; Isothiocyanates; Storage; Cooking; Freezing

## 1. Introduction

Epidemiological studies have shown that one of the major hydrolysis products of glucosinolates in *Brassica* vegetables, isothiocyanates, have protective effect against cancer – particularly in the bladder, colon and lung. In clinical studies of cancer preventive activity, there has been little information collated of the dietary intake of glucosinolates and isothiocyanates. This frequent failure

to quantify glucosinolate and isothiocyanate inputs is associated with several technical problems that have not been resolved completely: (i) the availability of validated analytical methods for the quantitation of the major glucosinolates and their degradation products in *Brassica* vegetables; and (ii) limited understanding of the effects of storage and culinary processing (preparation for cooking and cooking) on the stability of glucosinolates in *Brassica* vegetables. Consequently, dietary inputs of glucosinolates isothiocyanates and their relation to associated bioactivity have been difficult to characterise and control (Thornalley, 2002; Thornalley and IARC Workgroup, 2004).

The cancer chemopreventive effect of *Brassica* vegetables is linked to the level of glucosinolates, their conversion to isothiocyanates and bioavailability of the isothiocyanate metabolite. Storage and culinary processing of vegetables

*Abbreviations:* ESP, epithiospecifier protein; LC–MS/MS, liquid chromatography with tandem mass spectrometric detection; LOD, limit of detection; MRM, multiple reaction monitoring.

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may affect the glucosinolate and related isothiocyanate contents of the *Brassica* vegetables commonly consumed. Vegetables are usually purchased from a local store or farm and stored at 4–8 °C in a refrigerator or at ambient temperature for up to a week. Glucosinolates are chemically stable until they come in contact with the enzyme myrosinase, which is stored compartmentalised from glucosinolates in plant tissue (Kelly et al., 1998). They become accessible to myrosinase when the plant tissue is disrupted, such as in insect predation or when vegetables are frozen and thawed, chopped and shredded in preparation for cooking (Quinsac et al., 1994; Rodrigues and Rosa, 1999; Shapiro et al., 2001; Verkerk et al., 2001).

Culinary processing has complex influences on the nutrient and non-nutrient content of vegetables (Holst and Williamson, 2004; Rock et al., 1998). Cooking at high temperatures denatures myrosinase in vegetable material, resulting in a lower conversion of glucosinolates to isothiocyanates when chewed. Glucosinolates of consumed vegetables are degraded to isothiocyanates by myrosinase of gut bacteria (Shapiro et al., 2001). The effect of cooking

on glucosinolates has been investigated but reports give conflicting evidence (Dekker et al., 2000; Smith et al., 2003; Vallejo et al., 2002; Verkerk et al., 1997; Verkerk and Dekker, 2004).

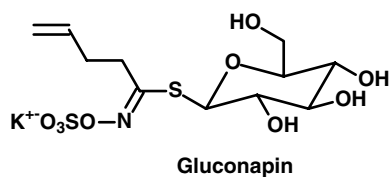
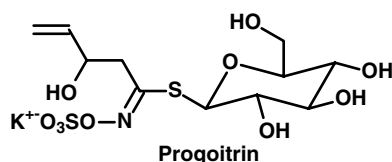
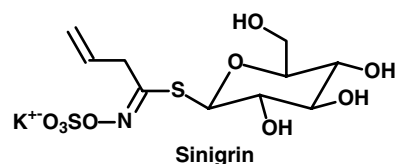
In this work, we report the effect of storage, processing and cooking methods on the glucosinolate contents in four *Brassica* vegetables. LC–MS/MS has been employed for the quantitation of glucosinolates, isothiocyanates and amine degradation products.

## 2. Materials and methods

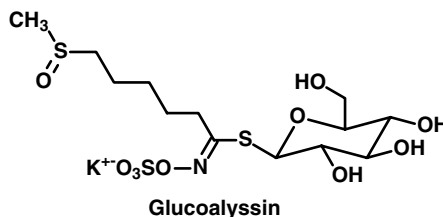
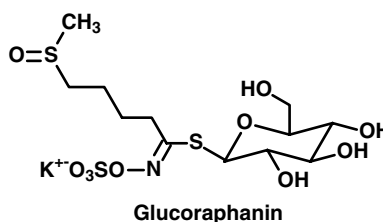
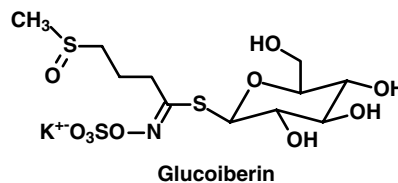
### 2.1. Materials

*Brassica* vegetables, broccoli, Brussel sprouts, cauliflower and green cabbage, were purchased from a local store. They were free from insect and mechanical damage. The vegetables were transported to the laboratory within 30 min of purchasing. Analytical standard glucosinolates and related isothiocyanate and amine degradation products were prepared and purified as described (Song et al., 2006). Mass spectrometric analysis was performed using a Waters Alliance 2695 HPLC system with a Micromass Quattro Ultima triple quadrupole mass spectrometer (Waters-Micromass, UK) (Fig. 1).

#### Olefinic glucosinolates:



#### Methylsulfinylalkyl glucosinolates:



#### Aryl glucosinolate:

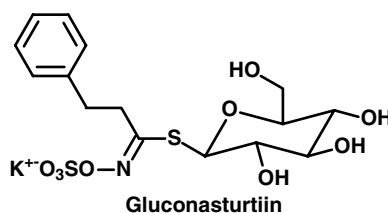


Fig. 1. Molecular structure and classification of glucosinolates studied in this work. The potassium salt form is given.

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