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Development of a method for producing selenium-enriched radish sprouts

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Se
Selenium
SE
Sinigrin equivalents
FW
Fresh weight
DW
Dry weight
HPLC
High performance liquid chromatography
LCMS
Liquid chromatography-mass spectrometry
PDA
Photodiode array

ABSTRACT

Brassicaceae is one of the few plant families with the ability to incorporate the essential trace element selenium (Se) into organic compounds, and the vegetables are claimed to have a number of human health benefits based on both Se and glucosinolate content. We investigated the effect of Se addition on the nutrient composition of broccoli, purple radish and green radish sprouts and develop an efficient growing method for producing high concentration Se radish sprouts that does not impact the glucosinolate content and has little seleniferous waste.

The concentration of Se in sprouts increased exponentially with time in the presence of high Se supply (9.2 μmol Se g^{-1} seed). At lower rates of Se supply ($\leq$2.5 μg Se g^{-1} seed) sprouts containing a target Se concentration were produced according to the linear relationship $y = 0.91x$, where y is the target Se concentration in μg Se g^{-1} FW, and x is the concentration of sodium selenate in water added at 8.3 mL of water g^{-1} dry seed. Glucosinolate profiles differed between broccoli and radish but the total glucosinolate concentration of the sprouts was unaffected by Se addition. Radish was more suitable than broccoli for producing sprouts high in both glucosinolates and Se.

1. Introduction

Selenium (Se) is an essential trace element for humans (Kieliszek & Blazejak, 2013; Sunde, 1997) but Se intakes are low in countries where soil Se concentrations are low, such as New Zealand, Finland, the United Kingdom and Germany (Broadley et al., 2006; Thomson, 2004). In addition to its involvement in cellular protection against excess peroxide, and the proper functioning of the immune system and thyroid gland (Kieliszek & Blazejak, 2016), some health studies suggest that Se may have anti-cancer properties (Kieliszek & Blazejak, 2016; Whanger, 2004). However, Klein et al. (2011) found that oral Se, as selenomethionine, did not reduce the likelihood of men developing prostate cancer. Finley et al. (2001) found that increased Se (as selenite) in the diet reduced the risks of rats developing precancerous crypts, and that Se was much more effective when provided in plant tissue such as broccoli florets or sprouts where it was likely to have been present as Se-methylselenocysteine (Abdulah et al., 2009; Cai et al., 1995). Abdulah et al. (2009) found that Se-enriched broccoli sprouts were superior to normal broccoli sprouts in inducing programmed cell death of prostate cancer cells and only Se-enriched broccoli sprouts induced a down-regulation of the Akt/mTOR pathway – a pathway that is overactive in cancerous cells.

These reported health benefits have given rise to an increased interest in the biofortification of plants with Se (Mora et al., 2015). An important factor to consider in commercial production of Se-fortified food is that while Se is essential for animals in trace concentrations, at high concentrations it is extremely toxic to a wide range of organisms (Debruyn & Chapman, 2007; Koller & Exon, 1986) and becomes an environmental pollutant. It is important, therefore, that the added Se is taken up by the plant, with minimal Se requiring disposal or discharge into the environment. Another issue with Se enrichment of plants for human consumption is the disposal of the waste plant material. In the case of Se-enriched tomato plants, the vine accounts for a significant amount of biomass and can contain Se concentrations one or more orders of magnitude higher than those found in the fruit (Brummell et al., 2011). An advantage with biofortifying sprouts is that all of the

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plant material is eaten, apart from the seed husk, so there is very little waste. An additional advantage of consuming Se in brassicas (and alliums), as opposed to inorganic supplements and many other plant species, is that these plants convert much of the Se to selenocysteines (Abdulah et al., 2009; Pyrzynska, 2009), which are reported to be less toxic than inorganic forms and selenomethionine (Bueno, Pannier, & Potin-Gautier, 2007).

In addition to the benefits claimed for Se-enrichment, brassicas also contain glucosinolates, which are natural sulphur-rich compounds. Glucosinolates are claimed to have anti-oxidant activity (Cabello-Hurtado, Gicquel, & Esnault, 2012), particularly when broken down by enzymes to isothiocyanates and these compounds have been linked to potential anti-cancer effects (Lenzi, Fimognari, & Hrelia, 2014). Broccoli sprouts contain high concentrations of glucosinolates compared with mature broccoli heads (Tian, Rosselot, & Schwartz, 2005); and some brands of broccoli sprouts, e.g. Broccosprouts® (www.broccoprouts.com), are marketed on glucosinolate content.

Selenium fertilisation (biofortification) may affect glucosinolate production in *Brassica* spp. For instance, Finley, Sigrid-Keck, Robbins, and Hintze (2005) reported Se fertilisation reduced glucosinolate production in mature broccoli, whereas Schiavon et al. (2016) showed Se addition increased glucosinolate production in radish roots. Sams, Panthee, Charron, Kopsell, and Yuan (2011) reported that Se addition (as selenate) influenced the expression of a number of genes involved in the production and degradation of glucosinolates in *Arabidopsis*.

It appears that the effect of Se addition on glucosinolate concentration during sprout growth may differ to older plants, and may vary for different *Brassica* spp. Avila et al. (2014) added selenate to a range of *Brassica* sprouts and found that the concentrations of various glucosinolates were largely unaffected in broccoli, green cabbage and Brussels sprouts, but there was some effect of selenate addition on the concentration of glucosinolates in cauliflower, Chinese cabbage and kale. There appear to be no studies that have investigated the effect of selenate addition on the glucosinolate profile of radish sprouts. Here, we investigate the glucosinolate concentration and profile of purple radish sprouts, and compared the profiles with the more common green radish sprouts and broccoli sprouts, with and without Se treatment to establish whether Se treatment impacts glucosinolate concentrations.

While both broccoli and radish sprouts are grown commercially, to our knowledge, there are no commercially available brassica sprouts that are fortified with Se. Here, we describe the science necessary to underpin the development of this novel product, which requires understanding the relationship between Se supply and Se concentration in broccoli and radish sprouts. The research was designed to be applicable to commercial production of Se enriched sprouts, and a commercial practice for sprout production (not enriched with Se) was used as the basis for method development. The method was then adapted to produce a more reliable product with less Se waste. The target sprout Se concentration for this research was 35% of the RDI, based on the New Zealand Ministry of Health (NZ MoH) recommended dietary intake (RDI) of Se for an adult male is 70 μg Se day^{-1} (NHMRC, NZMH, & ADHA, 2006, pp. 221–227), i.e. 24.5 μg Se in a 4.5 g serve of sprouts (or 5.4 μg Se g^{-1} FW). This level of biofortification allows a 10% buffer to ensure that the sprouts meet the target of 25% RDI to make a health claim pre-approved by the NZ MoH as a "good source" of Se (ANZFSC, 1996). It was decided not to supply more Se, because diets will already contain some Se (Thomson, 2004) and the recommended maximum daily intake of Se is only 6 times the RDI–400 μg day^{-1} (WHO, 1996).

2. Materials and methods

2.1. Growing conditions

2.1.1. Experiment 1a: effect of Se concentration on broccoli sprouts

Fifteen gram samples of open-pollinated broccoli (*Brassica oleracea* L. var. italica Plenck) seed were soaked for 4 h (the length of time used in commercial practice) in 30 mL of one of six solutions of sodium selenate: 0, 2, 4, 8, 12, 20 mM Se. This was done in triplicate, giving 18 samples. The selenate solution was then drained off into a container for disposal. The seeds were rinsed twice in UV sterilised water then grown in the dark for 4 days at 21 °C in transparent 400 mL containers, with needle holes in the lid to allow aeration. Sprouts were rinsed daily with sterile water then harvested as described below. Note that the 0 Se treatment is based on a commercial practice in New Zealand.

2.1.2. Experiment 1b: effect of Se concentration on purple radish sprouts

Three gram samples of purple radish (*Raphanus sativus* L. cv. 'Sango') seed were soaked for 4 h in 5 mL of one of eight solutions containing sodium selenate (0, 1, 2, 5, 10, 20, 30, 40 mM Se) and sodium hypochlorite (2000 μg mL^{-1} Cl). This was done in duplicate, giving 16 samples. The selenate solution was then drained off, and the seeds rinsed three times in UV sterilised water. The sprouts were grown in the dark for 4 days at 20 °C and then harvested as described below.

2.1.3. Experiment 2: effect of soak time on sprout Se concentration

Sixty five grams of purple radish seeds were surface sterilised with sodium hypochlorite, then rinsed twice with UV sterilised water. The seeds were then soaked for up to 48 h in 2 L of 300 μM sodium selenate (solution to seed ratio of 31:1 mL g^{-1}) that was aerated at 21 °C. After 0, 4, 8, 16, 24 and 48 h triplicate samples were removed and grown in small transparent plastic pots in an incubator at 21 °C. Sprouts were then grown in the dark, then harvested 4 days after the seeds were first wetted, as described below.

2.1.4. Experiment 3: the effect of plant type and Se biofortification on sprout glucosinolate concentration

The glucosinolate concentrations of Se-enriched sprouts produced using the new method developed (a 48 h soak plus the addition of 300 μM Se, Experiment 2), were compared with sprouts produced using the commercial method (see the 0 Se method in experiment 1) These were analysed for glucosinolate concentration (as described below). The three plant types tested were open-pollinated broccoli sprouts (*Brassica oleracea* L.), purple radish sprouts (*Raphanus sativus* L. cv. 'Sango') and green radish sprouts (*Raphanus sativus* L.), and there were three replicates of each plant type.

2.1.5. Experiment 4: optimising the Se-biofortification methodology to reduce waste

The Se-biofortification methodology was optimised to reduce waste for commercial production. In brief, the differences from the previous methods were that the sprouts were grown at a lower Se concentration than in previous experiments, using less water, and the sprouts were exposed to the Se-enriched water for the entire 4 d growth period.

Three grams of purple radish seeds were weighed into 18 plastic containers (500 mL with a 3 mm diameter aeration hole, Fig. 1). The seeds were sterilised by soaking for 20 min in 50 mL of 2000 μg mL^{-1} sodium hypochlorite solution. After 20 min the seeds were drained and

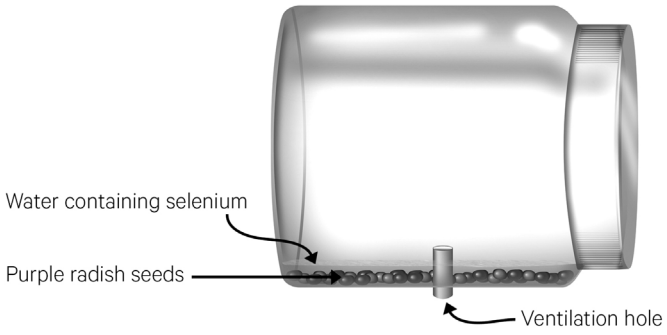

Fig. 1. Cross section of the sprout growing container.