

Supplementation of the diet by exogenous myrosinase via mustard seeds to increase the bioavailability of sulforaphane in healthy human subjects after the consumption of cooked broccoli

Article

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1 **Supplementation of the diet by exogenous myrosinase via mustard seeds to increase the**
2 **bioavailability of sulforaphane in healthy human subjects after the consumption of**
3 **cooked broccoli**

4
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14
15 **Shortened title: Increasing sulforaphane bioavailability from broccoli through exogenous**
16 **myrosinase.**

31 **Abstract**

32 **Scope**

33 Broccoli contains the glucosinolate glucoraphanin which, in the presence of myrosinase, can
34 hydrolyse to the isothiocyanate sulforaphane, reported to have anti-carcinogenic activity.
35 However, the myrosinase enzyme is denatured on cooking. Addition of an active source of
36 myrosinase, such as from powdered mustard seed, to cooked *brassica* vegetables can increase
37 the release of health beneficial isothiocyanates, however this has not previously been proven
38 *in-vivo*.

39 **Methods and results**

40 The concentration of sulforaphane metabolite (sulforaphane N-acetyl-L-cysteine (SF-NAC) in
41 12 healthy adults after the consumption of 200g cooked broccoli, with and without 1 g
42 powdered brown mustard, was studied in a randomized crossover design. During the 24 hour
43 period following consumption of the study sample all urine was collected. SF-NAC content
44 was assayed by HPLC. When study subjects ingested cooked broccoli alone, mean urinary SF-
45 NAC excreted was 9.8 ± 5.1 μmol per g creatinine, whilst when cooked broccoli was consumed
46 with mustard powder this increased significantly to 44.7 ± 33.9 μmol SF-NAC per g creatinine.

47 **Conclusion**

48 These results conclude that when powdered brown mustard is added to cooked broccoli the
49 bioavailability of sulforaphane is over four times greater than that from cooked broccoli
50 ingested alone.

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55 **Keywords: Mustard, broccoli, isothiocyanate, urine, myrosinase, bioavailability**

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66 **1. Introduction**

67 Globally, there is an increasing awareness of diet in maintaining good health and the prevention
68 of diseases such as diabetes, cancer and cardio-vascular diseases. Increased consumption of
69 vegetables is known to provide a variety of micro- and phytonutrients, which might have
70 beneficial effects on health [1]. *Brassica* vegetables provide micronutrients such as folate, and
71 phytochemicals such as glucosinolates. The later have been reported to possess potential anti-
72 carcinogenic effects, as well as improving risk factors for cardiovascular diseases [2-4].

73 Broccoli (*Brassica oleracea* var. *Italica*) contains glucoraphanin as its predominant
74 glucosinolate. Under favourable conditions, glucoraphanin is hydrolyzed either by plant
75 myrosinases (co-existing in different segregated parts of the same plant alongside the
76 glucosinolates) or by bacterial thioglucosidases (in human gut microflora) into the
77 corresponding isothiocyanate, sulforaphane (SF) (4-methylsulfinyl butyl isothiocyanate) and a
78 variety of other compounds depending on factors such as pH, metal ions and other protein
79 elements [5].

80 *Brassica* are subjected to various methods of processing, including cooking, prior to
81 consumption. Irrespective of the cooking methods, myrosinase will be denatured to varying
82 extents, however, the glucosinolates are rarely affected by cooking if leaching out can be
83 prevented [6-9]. Dekker et al., [9] pointed out that cooking *Brassica* vegetables alters the
84 glucosinolate - myrosinase system due to a number of factors, amongst which are; partial or
85 total inactivation of myrosinase, thermal/plant myrosinase mediated breakdown of
86 glucosinolates, loss of enzymic cofactors, leaching of glucosinolates and their metabolites into
87 the cooking medium, volatilization or thermal degradation of the metabolites. When
88 myrosinases are inactivated during *Brassicaceae* processing, the production of beneficial
89 health compounds is greatly diminished.

90 Most *Brassica* vegetables owe their chemo-preventive potentials to the presence of
91 glucosinolates, the subsequent conversion of these glucosinolates to isothiocyanates, and
92 bioavailability of the isothiocyanate metabolite [10]. SF is known to be an exceptionally active
93 inducer of phase II enzymes [11]. SF is metabolized by the mercapturic acid pathway,
94 predominantly appearing in urine as sulforaphane N-acetyl-L-cysteine (SF-NAC). SF is rapidly
95 absorbed, metabolized, and excreted, with 80% appearing in the urine within 12-24 hours after

96 consumption [12], although its bioavailability and overall therapeutic benefit may be affected
97 by pharmacokinetic properties, genetic variation, and food preparation [13]. SF-NAC is often
98 used as a marker of SF bioavailability, although it is not the only metabolite present in urine
99 [11, 12, 14-16].

100 Conaway et al., [17] investigated the metabolic fate of glucosinolates in humans after ingestion
101 of steamed and raw broccoli, and reported that the excretion of urinary isothiocyanate
102 metabolites was approximately 3 times greater in raw broccoli than from steamed broccoli. In
103 broccoli sprouts, Shapiro et al., [16] concluded that the availability and excretion of
104 glucosinolate was dictated by its conversion to isothiocyanates via myrosinase. Clarke et al.,
105 [13] concluded that the bioavailability of SF and erucin was lower in human subjects who
106 consumed broccoli supplements compared to fresh broccoli sprouts. The same authors
107 suggested that the food matrix, cooking, co-ingestion of other factors or the presence of proper
108 enzymes for metabolism can affect the bioavailability of bioactive dietary constituents derived
109 from *Brassica* vegetables [13, 18].

110 In some recent studies, the addition of an exogenous form of myrosinase isoenzymes to
111 processed *Brassicaceae* has been shown *in vitro* as capable of re-initiating glucosinolate
112 hydrolysis with the subsequent increase in beneficial hydrolysis products particularly SF [19,
113 20]. However, this increase in availability of SF had not yet been shown *in vivo* from such
114 studies.

115 The hypothesis of this study was that the addition of brown mustard powder would improve
116 the formation of SF when added to cooked broccoli because of the action of mustard
117 myrosinase on the broccoli glucoraphanin *in situ* (i.e. either immediately before consumption,
118 or during mastication and digestion). This study aims to investigate the bioavailability of the
119 SF, by measuring SF-NAC in human urine, after the consumption of cooked broccoli with and
120 without powdered brown mustard (*Brassica juncea*) by 12 healthy adults in a crossover design.
121 This is not only important for the investigation of potential health effects, but also for the
122 suitability of SF-NAC as a potential biomarker of intake.

123 **2. Materials and methods**

124 **2.1 Study design**

125 The study was given a favourable opinion for conduct by the University of Reading Research
126 Ethics Committee (study number UREC 15/30). Twelve non-smoking apparently healthy
127 adults, age 18-64 years, were recruited to attend the Hugh Sinclair Human Nutrition Unit,
128 University of Reading. All adult subjects read and understood study information sheet,

129 completed a health and lifestyle questionnaire and signed an informed consent document prior
130 to taking part in the study. Each subject attended a screening visit where physical examination,
131 complete medical history and food preferences were taken. Volunteer exclusion criteria
132 included: sufferers of chronic illnesses, individuals with food allergies and individuals who
133 disliked *Brassica* vegetables. Two intervention visits were carried out in a randomized
134 crossover study design, where participants were fed 200g cooked broccoli (*Brassica oleracea*
135 var. *Italica*) with and without 1g powdered brown mustard seeds (*Brassica juncea* (L). *Czern*),
136 with a 7-day washout period in-between the two visits. All 12 subjects screened completed
137 both study intervention days.

138 **2.1.1 Sample preparation**

139 Freshly harvested matured broccoli (*Brassica oleracea* var. *Italica*) grown in the UK was
140 purchased from Produce World Marshalls (Boston, UK). All broccoli used for the study was
141 obtained 24 hours prior to sample preparation and refrigerated (4 °C). Brown mustard (*Brassica*
142 *juncea* (L). *Czern*) seed, obtained from IPK Genebank (Gatersleben, Germany), was cultivated
143 in a glasshouse at the University of Reading, and harvested in the pod after maturation. The
144 harvested mustard seeds were allowed to dry in the pod under room temperature (18-23 °C)
145 and then manually shelled. The dried brown mustard was ground using a coffee blender, sieved
146 (30 µ mesh), stored in air-tight containers and refrigerated (4 °C). This was done 24 hours prior
147 to consumption.

148 On study day, the broccoli was washed and allowed to drain. The broccoli heads were cut
149 approximately 4 cm from the top, thoroughly mixed together and 200 g portions were weighed
150 into low density polyethylene (LDPE) bags and vacuum sealed. Each weighed portion was then
151 cooked *sous vide* in boiling water at 100 °C for 20 minutes. Cooking broccoli under this
152 condition for 10 minutes is sufficient to completely inactivate myrosinase enzymes as
153 established in our previous studies [18]. The cooked portion of broccoli was allowed to cool
154 and then pureed using a Kenwood Multi Pro FDP 613 blender (Kenwood, UK) with the
155 addition of 100 ml of potable water (25 °C). The pureed broccoli was then served to subjects
156 (25 °C) with and without the addition of 1g brown powdered mustard seed. Where mustard
157 powder was added this was done immediately prior to serving.

158 **2.1.2 Study sample administration and urine collection**

159 All study participants were asked to abstain from glucosinolate or isothiocyanates containing
160 foods (a list of excluded foods was given to each participant at recruitment and stated on the
161 study questionnaire), 48 hours prior to study visit day. Participants came in on the morning of

162 visit day fasted (no food and liquids other than water) from 8 pm of the evening preceding the
163 visit day. Participants attended the Hugh Sinclair nutrition unit before 10 am. Each participant
164 consumed 200 g of cooked broccoli, with or without 1 g powdered brown mustard powder
165 (with supervision), according to the study design. Potable water was made available during
166 sample consumption period, which was about 10-20 minutes. Participants were then directed
167 to collect all urine for 24 hours after ingestion of study sample, in urine pots containing 0.5 g
168 ascorbic acid (as preservative). The urine pots were placed in bags containing ice packs. The
169 urine samples were returned on the morning of the following day. The urine volume was
170 recorded, three 15 ml aliquot were centrifuged, and then stored at -20 °C until analysis. During
171 study days participants avoided excluded foods. To enable control of food intake for the first
172 meal after the broccoli intervention, a calorie controlled lunch was provided. This contained
173 sandwiches, crisps and fruit juice. Participants were encouraged to eat these two hours after the
174 broccoli intervention and they were encouraged to drink water during the 24 hour experimental
175 period. Food consumed later in the study day was not controlled, other than that exclusion of
176 the prohibited glucosinolate-containing foods.

177 **2.2 Determination of sulforaphane in urine**

178 SF-NAC quantification in urine was evaluated as described by Conaway et al., [17] with some
179 modifications. 1 ml Urine was acidified with 10 µl 2 M HCL and frozen using dry ice. The
180 frozen samples were allowed to thaw to room temperature and then centrifuged (1000 g) for
181 10 minutes. The supernatant was filtered (0.45 µm), 10 µl was used for HPLC analysis by
182 Agilent HPLC-UV system (Agilent 1200, Manchester, UK) using a Nova-Pak C18 (4 µm)
183 reverse phase column (4.6 mm x 250 mm) (Waters, Elstree, UK) with a flow rate of 1 ml/min
184 and a wavelength of 365 nm. The mobile phase was 0.1% Trifluoro acetic acid in 9:1 water-
185 acetonitrile with flow rate 1 ml/min. A standard NAC (Sigma Aldrich, UK) conjugate of SFN
186 (Sigma Aldrich, UK) was synthesized [14]. Briefly, 123 mg of 0.4 mmol NAC was dissolved
187 in 6 ml of 50 % aqueous ethanol, the pH of the solution was then adjusted to 7.8 with 1 N
188 NaOH. 36 mg 0.2 mmol SF dissolved in 3 ml ethanol was added to the NAC solution and the
189 mixture was stirred at ambient temperature on dry ice. The solvent was evaporated and a
190 standard curve for urinary SFN-NAC was constructed by using the synthetic SFN-NAC
191 dissolved in urine (0.5–2.5 mM).

192 **2.3 Sulforaphane determination in broccoli samples**

193 SF in broccoli (raw, cooked and cooked with brown mustard powder) was analyzed as
194 described by Ghawi *et al.*, [19] with some modifications. 150 mg Lyophilized broccoli powder

195 was mixed with 1.75 ml de-ionized water in 2 ml eppendorf tubes and incubated at 30 °C for
196 5hrs. The mixture was then centrifuged (13,000 g, 10 min) and the supernatant collected. A
197 further 1 ml de-ionized water was then added to the mix and centrifuged (13,000 g, 10 min),
198 following which the supernatant was removed and the procedure repeated. Supernatants were
199 combined and filtered. 10 ml Dichloromethane was added to the combined supernatant,
200 vortexed for 1 min and then centrifuged (13,000 g, 10 min). The organic phase was collected
201 and this was repeated twice. The supernatants were combined and salted out using 10 g sodium
202 sulfate. This was then dried using a rotary evaporator at 37 °C. The dried sample was re-
203 dissolved in 0.7 ml acetonitrile and filtered (0.22 µm) before final injection onto HPLC for SF
204 quantification. HPLC-UV system (Agilent 1200, UK) with Nova Pak C18 (4 µm) reverse phase
205 column (4.6 mm x 250mm) was used. Flow rate was 1 ml/min at wavelength of 254 µm. The
206 solvent system consisted of 10 % acetonitrile in water increased linearly to 60% acetonitrile
207 over 22 min, then raised to 100 % acetonitrile in 4 min, finally, this was run isocratically using
208 100% acetonitrile for 4 min to purge the column. Column temperature was 30 °C with injection
209 volume at 10 µl. SF was quantified by standard calibration curves, using standard SF (Sigma
210 Aldrich, UK) in acetonitrile (10-1800 µg/ml). The linearity of the standard curves was
211 expressed in terms of the determination coefficients from plot of the integrated peak area versus
212 concentration of the standards.

213 **2.4 Determination of effect of cooking time on myrosinase inactivation in broccoli**

214 10 g portion of broccoli in vacuum sealed LDPE bags were cooked *sous vide* for different
215 preset times (2-14 minutes). The cooked portions were rapidly cooled on ice. Myrosinase
216 activity in cooked broccoli portions was then assayed as described and adapted by Okunade et
217 al, [21]. Briefly, the ground material is extracted on ice with buffer (Tris-HCl 0.2 M, pH 7.5
218 containing EDTA 0.5 mM, dithiothreitol 1.5 mM and 0.4 g polyvinylpolypyrrolidone) and the
219 protein in the filtered supernatant is precipitated with ammonium sulfate. The centrifuged pellet
220 is re-suspended in Tris-HCL buffer and extensively dialysed to remove excess ammonium and
221 sulfate ions. Following centrifugation, the supernatant was frozen (-80 °C), lyophilised, and
222 the resulting powder was stored at -20 °C. Myrosinase activity was measured according to the
223 coupled enzymatic procedure where the sample was added to a reaction mixture containing
224 NADP/ATP, hexokinase/glucose-6-phosphate dehydrogenase, and ascorbic acid (cofactor).
225 Following equilibration, sinigrin solution (0.6 M) was added. The change in absorbance due to
226 the formation of NADP was measured at 340 nm using a D-glucose determination kit was used
227 (R-Biopharm Rhone, Heidelberg, Germany). Myrosinase activity was determined from the

228 initial linear rate of increase in the curve of absorbance against reaction time. A standard
229 myrosinase enzyme (Sigma Aldrich, UK) was used to establish the calibration curve of
230 absorbance against concentration. One unit (un) of myrosinase was defined as the amount of
231 enzyme that produces 1 μmol of glucose per minute when sinigrin is used as a substrate at 25
232 $^{\circ}\text{C}$ and pH 7.5.

233 **2.5 Determination of creatinine in urine**

234 Creatinine was determined using ILAB 600 clinical chemistry analyzer (Instrumentation
235 Laboratories Werfen, Warrington, UK) and creatinine standard. Urine samples were defrosted
236 at room temperature, vortexed (Whirl mixer, Fisons, UK) and centrifuged at 1,500 g for 5 min.
237 150 μl of centrifuged sample was then pipetted into a labelled 3 ml sample cup, ensuring that
238 there were no air bubbles present in the sample. The standard creatinine was loaded in the test
239 reagent compartment while urine samples were loaded into the sample compartment of the
240 equipment. The samples were then analyzed after replacing the lid on the reagent and sample
241 compartments, ensuring that the lid was in the correct position.

242 **2.6 Data analysis and statistics**

243 For both urine and broccoli samples, the mean (\pm SD) SF concentrations of triplicate analysis
244 are presented. Significant differences between the two broccoli interventions (with and without
245 mustard seed) were determined using two-way ANOVA where the intervention was a fixed
246 effect and the participant a random effect. Analysis was carried out using PASW Statistics 21,
247 IBM, UK.

248 **3. Results and discussion**

249 **3.1 Effect of cooking time on broccoli myrosinase inactivation**

250 The effect of cooking time on myrosinase inactivation in broccoli was studied (Figure 1). When
251 broccoli was cooked *sous vide*, it was observed that myrosinase activity decreased with
252 exposure time. This trend is similar to that observed in previous studies where gradual increase
253 in temperature led to marked decrease in myrosinase activity [19, 21-23]. Cooking broccoli for
254 2 minutes led to 40% loss in myrosinase activity. Further increase in cooking time to 6 minutes
255 increased activity loss (90%) and after 8 minutes of cooking, there was no measurable
256 myrosinase activity recorded. Broccoli myrosinase is temperature sensitive and its temperature
257 stability has been reported in the literature [24-27].

258 **3.2 Sulforaphane in raw broccoli, cooked broccoli and cooked broccoli with mustard 259 powder**

260 Table 1 shows the SF content in raw broccoli, cooked broccoli and cooked broccoli with 1g
261 mustard powder used for this study. The SF content of unprocessed broccoli was 2.05 ± 0.25
262 $\mu\text{mol/g}$ dry weight while that of the cooked (*sous vide* cooking) sample was significantly lower
263 $1.06 \pm 0.18 \mu\text{mol/g}$ dry weight. This was expected as broccoli myrosinase is sensitive to
264 thermal treatment and is inactivated on cooking which inhibits the formation of SF. However,
265 when broccoli is eaten raw, sulforaphane nitrile is the main hydrolysis product [28] and
266 processing broccoli at low temperatures (under $50\text{ }^{\circ}\text{C}$) encourages the formation of
267 sulforaphane nitrile [25] as epithiospecifier protein activity is prominent at low temperatures
268 [29]. However the same authors suggested that heating broccoli florets up to $60\text{ }^{\circ}\text{C}$ favours the
269 formation of SF.

270 The low concentration of SF in the raw and cooked broccoli samples was expected. However,
271 when 1 g brown mustard was added to the *sous vide* cooked broccoli sample (*in vitro*), there
272 was a significant 8 fold increase in SF content (to $8.58 \pm 0.13 \mu\text{mol/g}$ dry weight). This
273 indicates that the addition of brown mustard to cooked broccoli significantly improved the
274 conversion of intact glucosinolate in the cooked broccoli (in which the myrosinase had been
275 inactivated). Brown mustard myrosinase hydrolysed the intact glucosinolates in the cooked
276 broccoli thereby improving the formation of more SF. Mustard myrosinase is robust and
277 thermally stable compared to broccoli myrosinase [21, 23]. Recent research suggests
278 alternative cooking modifications can also lead to increased SF formation from cooked
279 broccoli. Soaking broccoli florets in water at 37°C for 90 minutes prior to stir frying was shown
280 to promote hydrolysis before myrosinase was denatured on stir frying, leading to a 2.8 fold
281 increase in SF [30].

282 **3.3 Sulforaphane-N-acetyl cysteine in human subject urine**

283 The amount of SF-NAC excreted in subject urine over 24 hours after the consumption of the
284 study sample is shown in Figure 2. Shapiro et al., [16] had earlier pointed out that the extent of
285 chewing before swallowing might influence the bioavailability of fresh unhydrolyzed broccoli
286 sprouts. This was taken into consideration for this study, hence, the cooked broccoli was made
287 into puree form with addition of 100 ml water. After the consumption of cooked broccoli alone,
288 subjects excreted a mean $9.8 \pm 5.1 \mu\text{mol}$ SF-NAC per g creatinine within 24 hours whereas
289 following ingestion of the cooked broccoli with 1g powdered brown mustard, they excreted
290 $44.7 \pm 33.9 \mu\text{mol}$ SF-NAC per g creatinine within 24 hours. Conaway et al., [17] described the
291 bioavailability of SF in raw and steamed broccoli in human subjects to be in the ratio 3:1. In
292 the present study, the concentration of SF-NAC in the urine following a meal where mustard
293 was added to cooked broccoli, to that of cooked broccoli alone, was on average in the ratio of

294 4.7:1. The relative increase in SF-NAC from the cooked broccoli alone meal to the cooked
295 broccoli with mustard meal varied between the individual subjects from 1.7 fold to 10 fold.
296 This indicates that adding brown mustard powder to cooked broccoli had a beneficial effect of
297 substantially increasing the formation of SF in all subjects (where SF-NAC was quantified as
298 the metabolite of SF). Due to the nature of the study, it cannot be proven whether the increase
299 in SF from hydrolysis of the broccoli glucoraphanin occurred before broccoli intake, during
300 mastication, or during digestion. However, it is clear is that the addition of mustard powder to
301 the cooked broccoli immediately prior to consumption enabled more effective conversion of
302 glucoraphanin to SF.

303 In the cooked broccoli, the myrosinase would have been largely inactivated [24], hence
304 glucosinolate would have remained predominantly intact and, therefore, the SF in the urine
305 post consumption of the standard cooked broccoli ($9.8 \pm 5.1 \mu\text{mol/g}$ creatinine) would have
306 resulted primarily from gut microflora conversion in the digestive tract. The action of human
307 gut microbiota can hydrolyse glucosinolates, however, the isothiocyanate yield is known to be
308 much considerably lower than that obtainable from plant myrosinase [17, 31].

309 In addition, the isothiocyanate produced by the action of the gut microflora might have
310 undergone further degradation leading to the formation of other compounds which reduces the
311 amount of isothiocyanate available for absorption [32, 33].

312 Broccoli myrosinase compared to myrosinase from mustard (*Brassica juncea*, *B. nigra* and
313 *Sinapis alba*) is known to be more temperature sensitive [24]. The powdered mustard seed
314 added to the cooked broccoli provided an active myrosinase source and, therefore rapidly
315 increased the formation of isothiocyanates. Brown mustard myrosinase is known to be more
316 robust with good thermal stability compared to broccoli myrosinase [21, 22]. On average, the
317 resulting amount of SF excreted in the urine (measured as SF-NAC) after consumption of
318 broccoli with mustard was 4.7 fold higher than that excreted from consumption of cooked
319 broccoli alone. The addition of 1g mustard to *sous vide* cooked broccoli (*in vitro*) (Table 1) led
320 to an increased SF content from 1.06 ± 0.18 to $8.58 \pm 0.33 \mu\text{mol/g}$ dry weight, an 8 fold increase
321 in SF content. The rate of increase when mustard was added to cooked broccoli *in vitro* was
322 higher than that observed from the *in vivo* study (8:1 for *in vitro* study and 4.7:1 for the human
323 study). This slightly lower conversion rate is perhaps expected, and may be associated with
324 conversion in the gut. According to Lampe [34], the major metabolic pathway of
325 isothiocyanates in humans is by conjugation with glutathione and these conjugates are
326 subjected to further degradation by enzymes to yield the final metabolites. In the case of the

327 human study (*in vivo*), unhydrolysed glucosinolate may have been degraded by human gut
328 microflora which could partly explain the difference in the relative results.

329 **4 Conclusion**

330 Broccoli myrosinase is relatively temperature sensitive and is inactivated when core
331 temperature exceeds 60 °C. From the results of the current study, it can be concluded that the
332 addition of powdered brown mustard to cooked broccoli greatly enhances the formation of SF
333 compared to the cooked broccoli in which the myrosinase isoenzymes has been inactivated,
334 confirming that the presence of plant myrosinase is important for SF bioavailability. When
335 brown mustard powder was added to cooked broccoli, SF formation was promoted (8-fold),
336 hence improving its bioavailability, and resulting in an increase in the metabolite sulforaphane
337 N-acetyl-L-cysteine (SF-NAC) of over 4-fold. However, this conclusion must be considered
338 within the context of both plant and human variability. Bioavailability of glucosinolates and
339 isothiocyanates can be influenced by plant storage, processing, genetic variation of the plant
340 and the food matrix. In addition, human genetic variation influences the bioavailability of SF.
341 Glutathione S-transferase (GST) metabolises isothiocyanates and lack of this enzyme is
342 associated with more rapid excretion of SF; hence individuals with this genetic variation may
343 derive less benefit from consumption of *Brassica* vegetables.

344 **Acknowledgements**

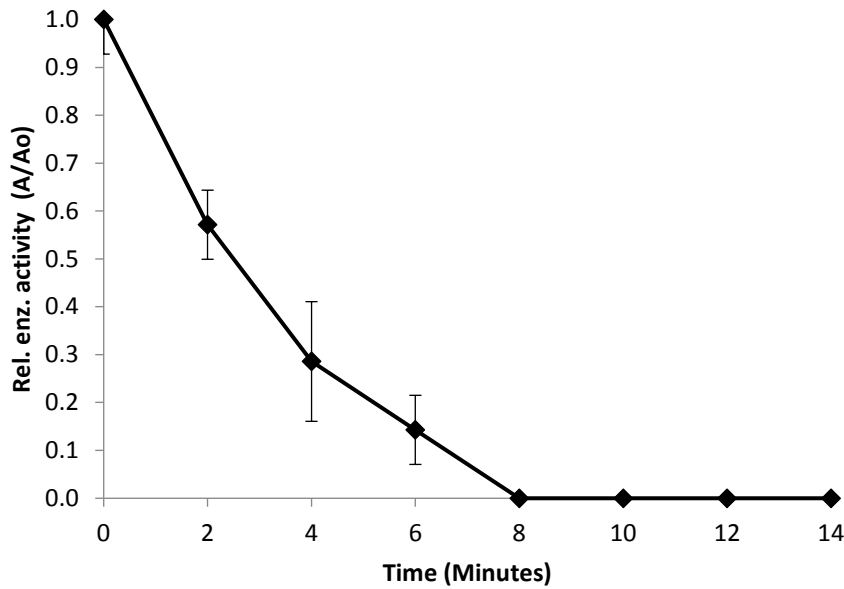
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348 providing the mustard seeds, and TETFUND Nigeria for sponsoring the first author.

349

350 **Figures and tables**

351 **Figure 1:** Effect of cooking time on myrosinase inactivation in *sous vide* cooked broccoli
352 (water temperature 100 °C). Error bars represent standard error of the mean.

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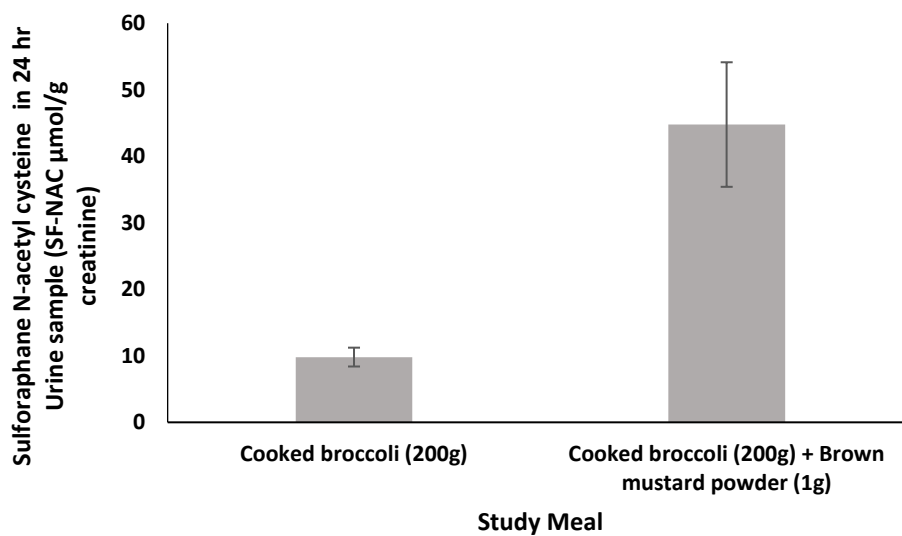
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357 **Figure 2:** Sulforaphane N-acetyl-L-cysteine (SF-NAC) /g creatinine excreted in 24 hours by
358 12 healthy adults (mean data). Error bars represent standard error of the mean.

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361

362 **Table 1:** Sulforaphane content of raw broccoli, cooked broccoli and cooked broccoli with 1g
 363 brown mustard powder added post cooking. Broccoli was cooked *sous vide* in boiling water at
 364 100 °C for 20 minutes

Sample	Sulforaphane ($\mu\text{mol/g}$ dry weight)*
Raw broccoli	2.05 \pm 0.25 ^b
Cooked broccoli	1.06 \pm 0.18 ^a
Cooked broccoli + 1g brown mustard powder	8.58 \pm 0.33 ^c

366 *Values not sharing a common letter are significantly different at $P < 0.05$.

367

368

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