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Novel approach to intensify the formation of the bioactive sulforaphane in cooked *Brassica* vegetables

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Glucosinolates-myrosinase system in *Brassica* vegetables

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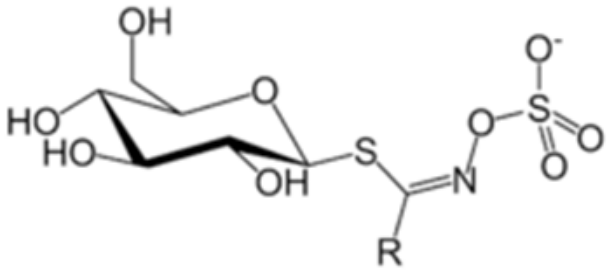
➢**Glucosinolates (GLS)**, sulphur and nitrogen-rich products**,** are a group of secondary plant metabolites found in Brassica vegetables.

➢To date, over 120 different glucosinolates have been identified (Oerlemans et al., 2005), categraizing is based on the side group (Fahey, Zalcmann, & Talalay, 2001)

➢**Myrosinase** (thioglucoside glucohydrolase) **:** is a family of enzymes found in all glucosinolate containing plants



Glucosinolate structure; side group R

Glucosinolates-myrosinase system in *Brassica* vegetables

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- In plant tissues, myrosinase and glucosinolates are physically segregated in distinct compartments. This cellular segregation keeps glucosinolates stable.
- Myrosinase catalyzes the hydrolysis of glucosinolates.
- Upon tissues damage during handling or processing of *Brassica* vegetables, myrosinase comes into contact with glucosinolates and the hydrolysis initiates.
- Hydrolysis releases glucose and **different compounds** depending on the side group of glucosinolates and the reaction conditions (pH, EPS, metallic ions).

Importance of the breakdown products

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- In addition to their **nutritional** value, *Brassica* vegetables are thought to have chemoprotective properties
- Some of the hydrolysis compounds (e.g. indole and isothiocyanates) are claimed to be **cancer-protective** compounds.
- Sulforaphane (isothiocyanate), derivative of 4-methylsulfinylbutyl glucosinolate (glucoraphanin), received a particular interest.
- Moreover, recent studies showed sulforaphane to exhibit **antimicrobial activity** against a wide variety of bacterial and fungal pathogens.
- Glucosinolates and the hydrolysis compounds contribute the distinct **aromas** and **tastes** in *Brassica* vegetables.

Glucosinolates-myrosinase system

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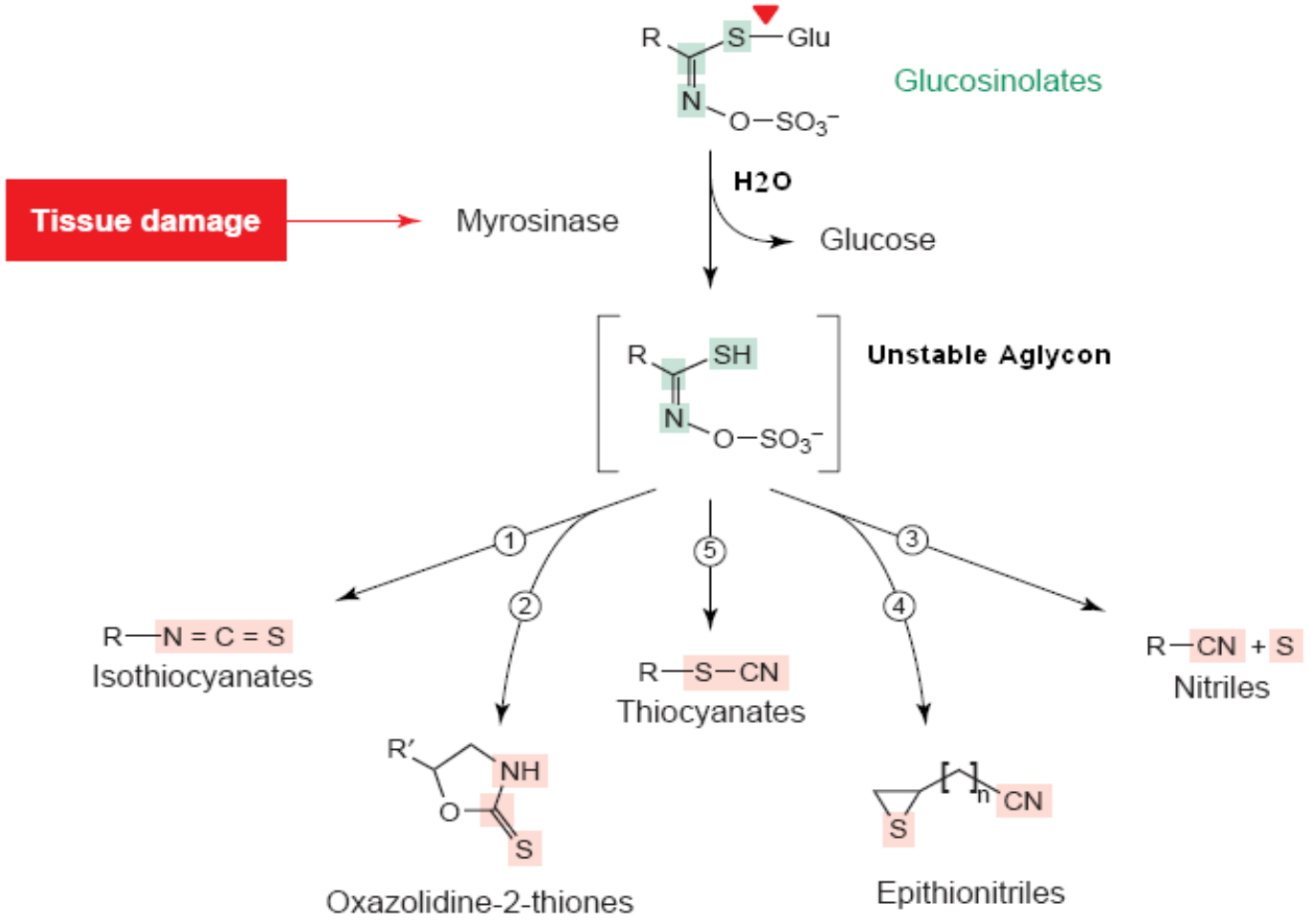

Glucoraphanin- myrosinase reaction

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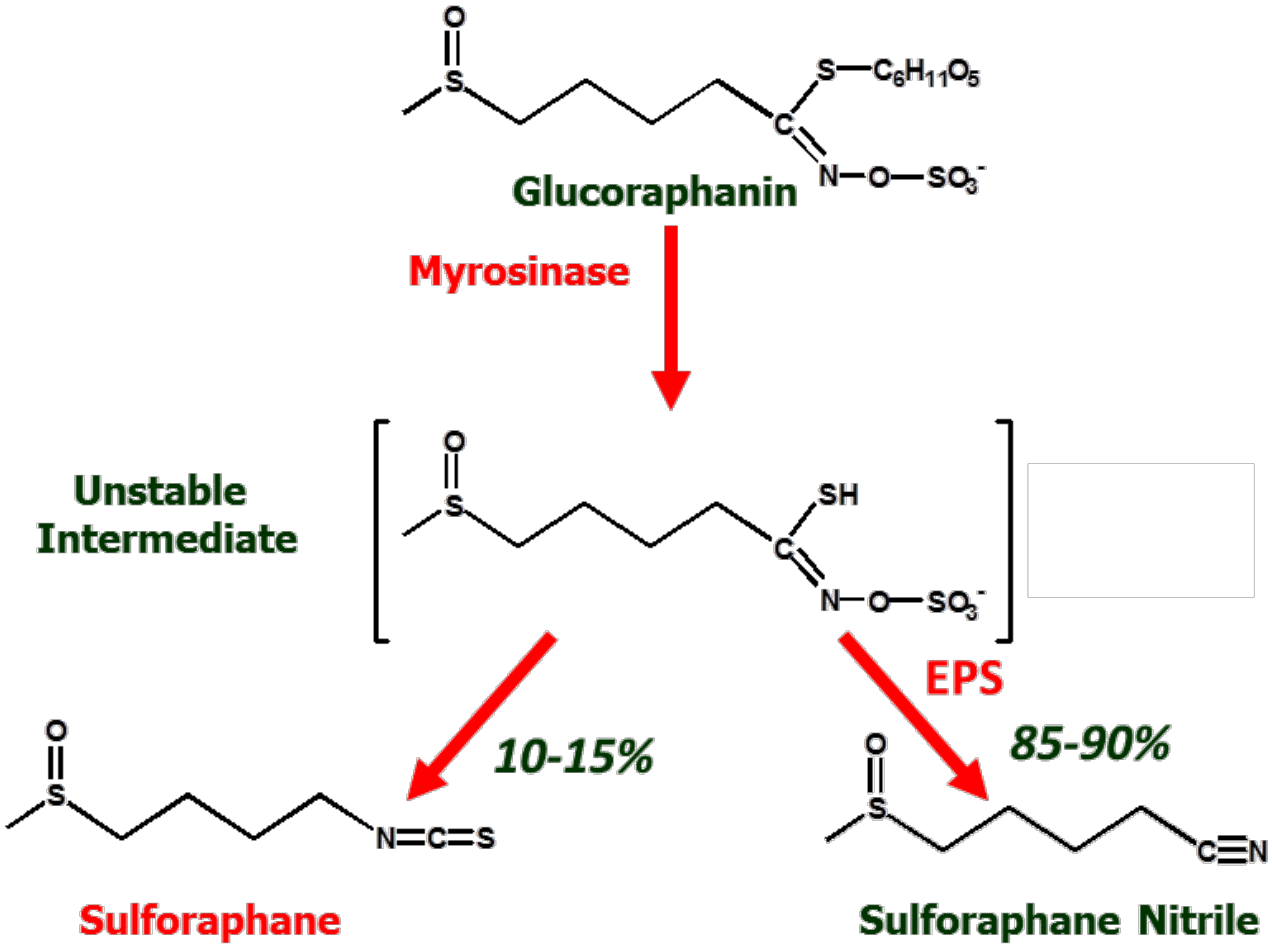

Thermal stability of glucosinolates-myrosinase system

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- Glucosinolates are thermally stable, and most of the decline in glucosinolates content after cooking is basically due to leaching into processing water.

- On the other side, myrosinase is more likely to be inactivated after domestic cooking, and hence stop of the hydrolytic breakdown of glucosinolates.

- This means consuming intact glucosinolates from cooked brassica.

Fortunately, glucosinolate hydrolysis may occur in the gastrointestinal tract under the action of the colonic microflora, however, the hydrolysis **rate is much lower** compared to hydrolysis resulting from the plant myrosinase (usually less than one third).

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Study approach

During my PhD project, I utilized two approaches to enhance the formation of sulforaphane.

The first was by applying HPP which I am not talking about today.

The second approach:

Addition of low concentrations of *Brassica* condiments to cooked broccoli, has thermally inactive myrosinase, will reinitiate the hydrolysis reaction of glucoraphanin towards sulforaphane.

These sources will provide an intact myrosinase, and might be used as a condiments to improve **palatability** of *Brassica* vegetables.

Mustard seeds as was used as additive in this study:

- Has higher thermal stability myrosinase.
- does not contain ESP as previously reported.
- Very widely used in diet.

Methods

Sample preparation

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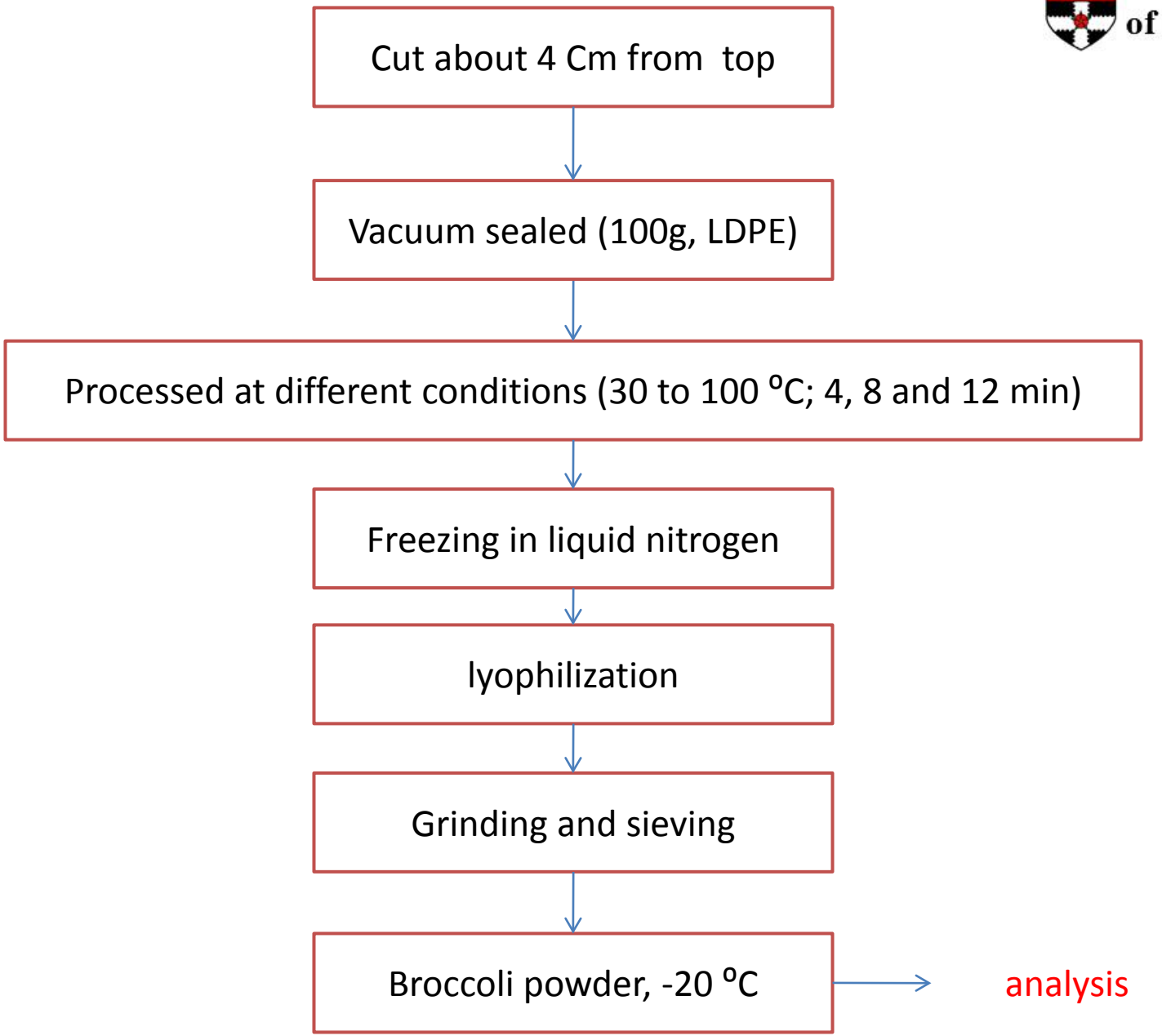

Methods

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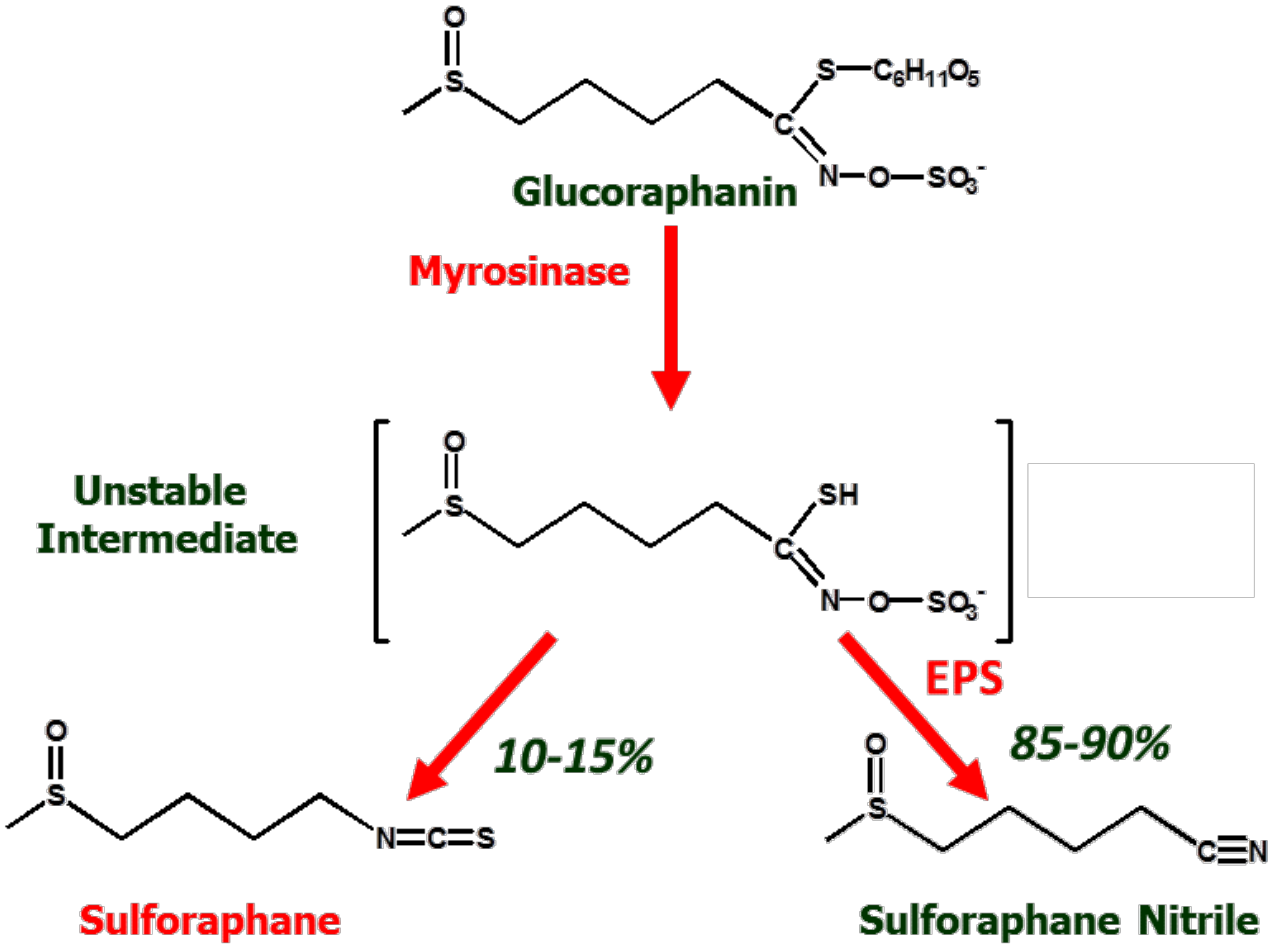

Myrosinase thermal stability

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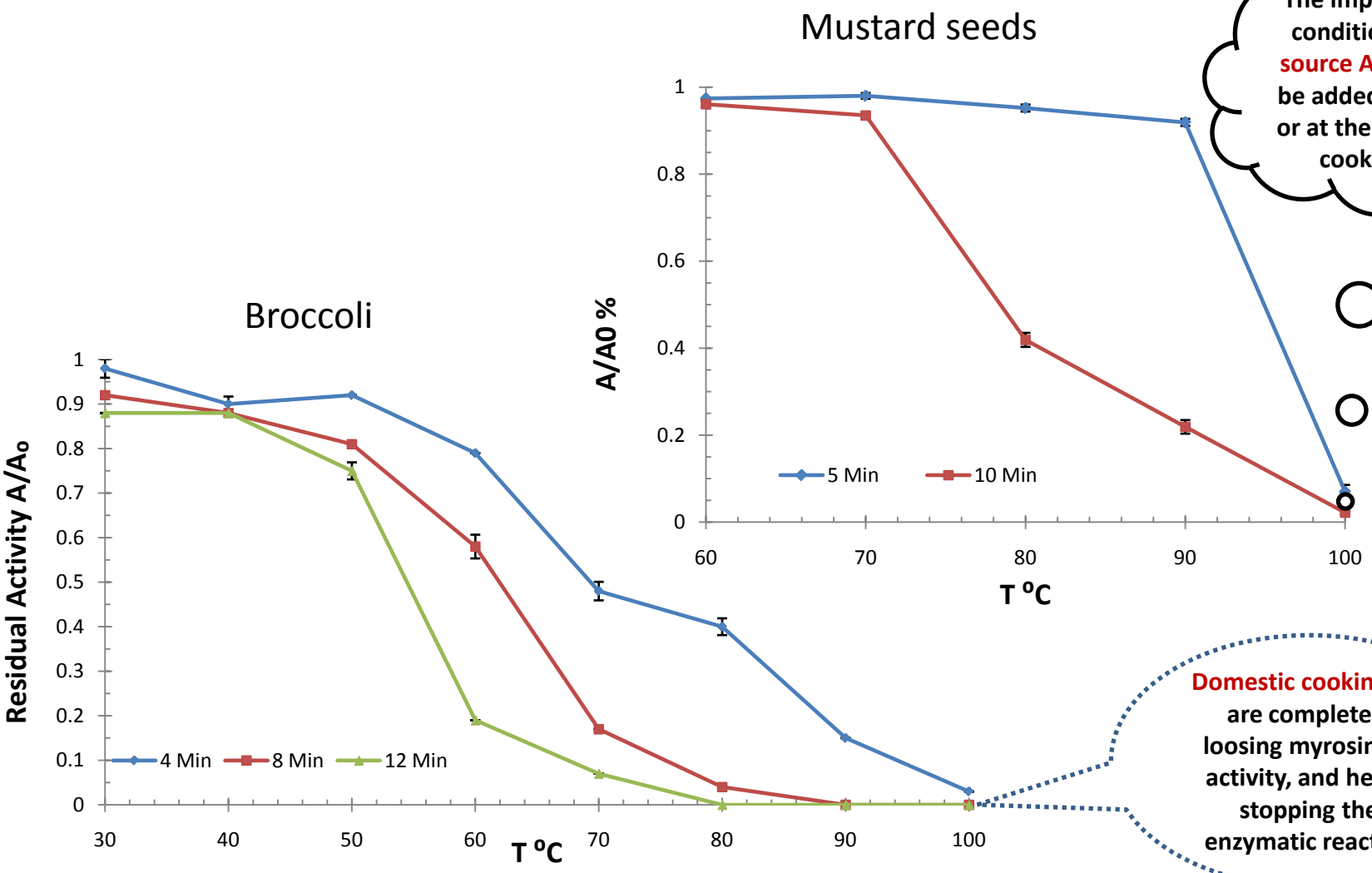
The important condition, as source A might be added while or at the end of cooking



Domestic cooking we are completely loosing myrosinase activity, and hence stopping the enzymatic reaction

Thermal stability of glucoraphanin in broccoli (no enzymatic reaction, no leaching)

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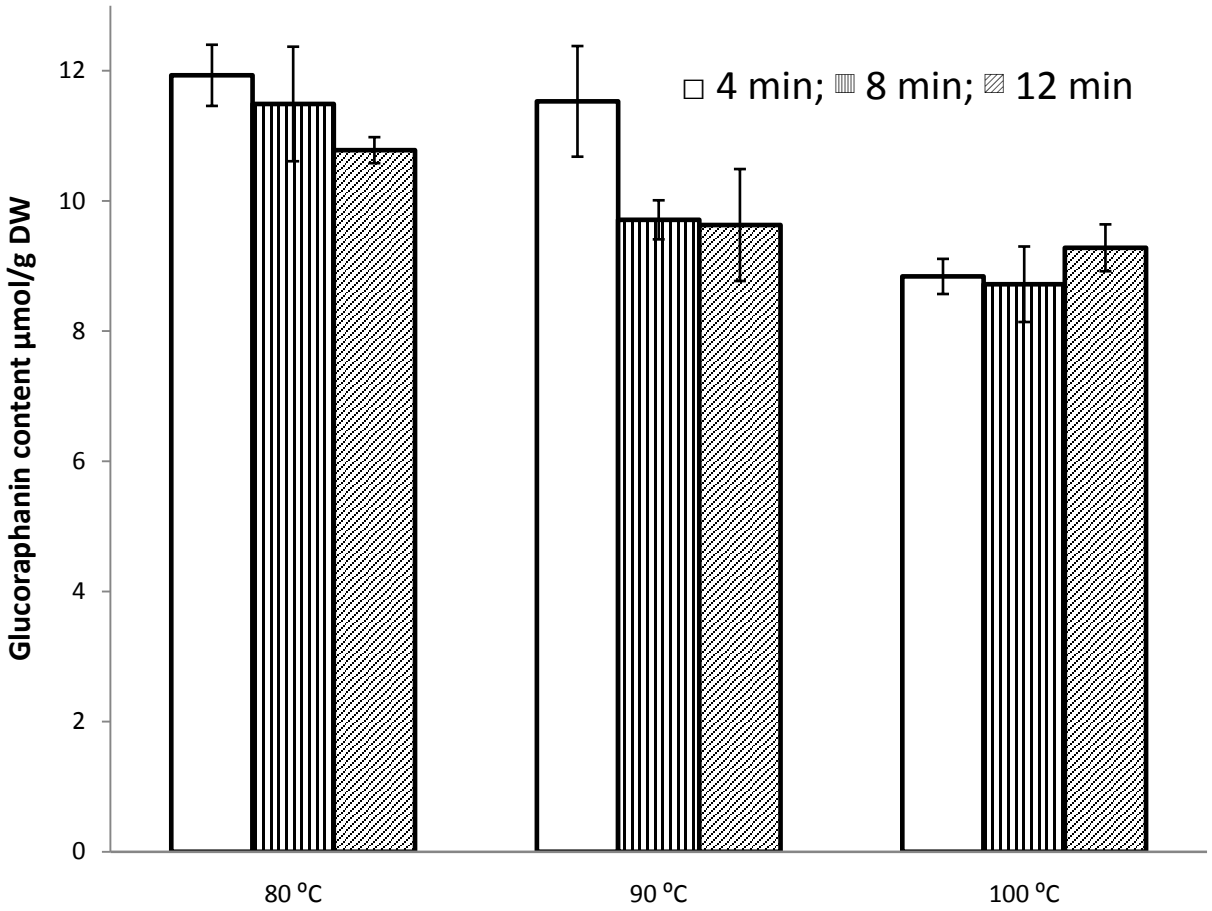
Glucoraphanin content in raw broccoli:10.3±0.21 µmol/g DW



Effect of thermal processing on sulforaphane content in broccoli

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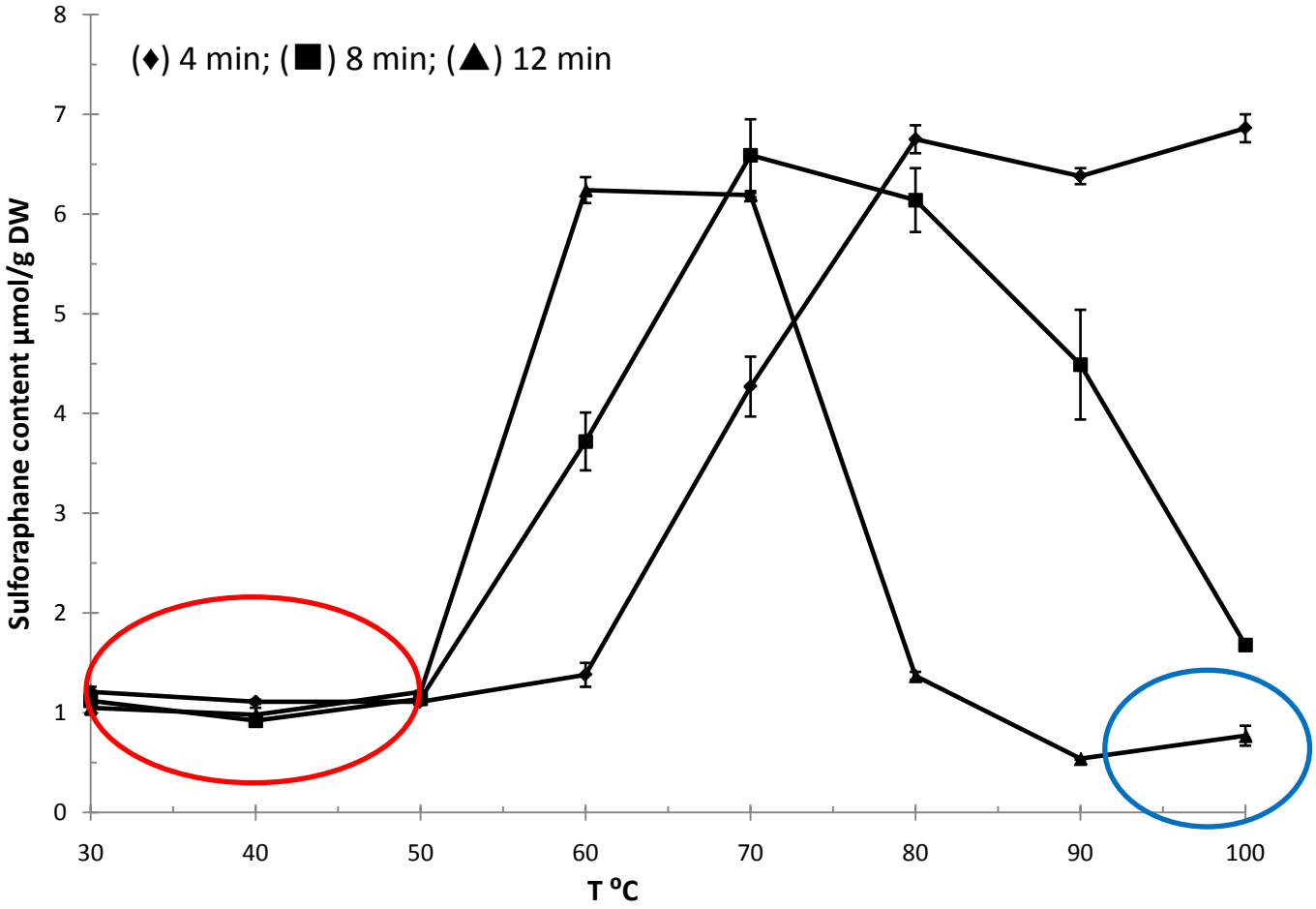

Effect of thermal processing on sulforaphane nitrile content in broccoli

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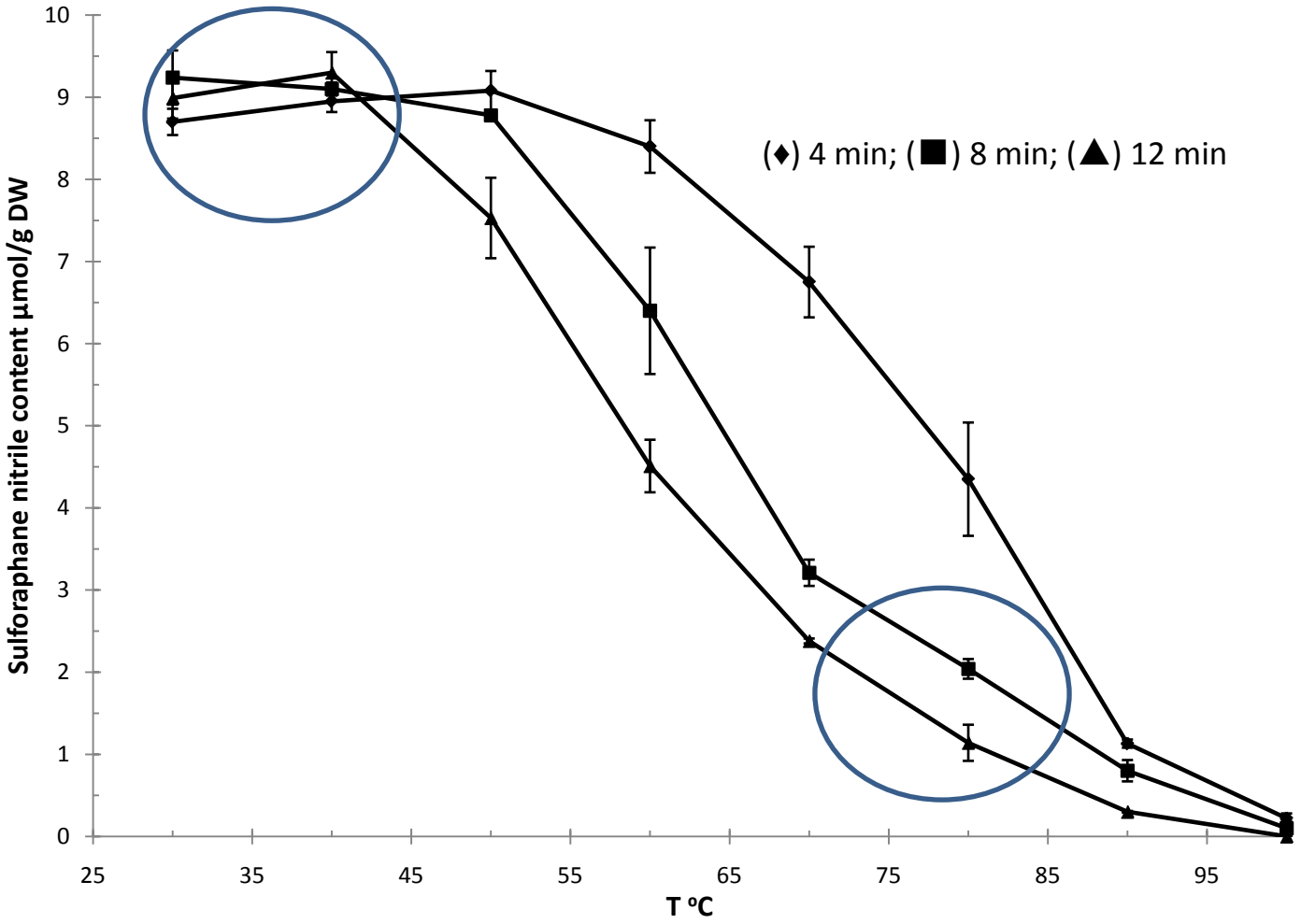

Sulforaphane content in cooked broccoli samples after adding mustard seed powder.

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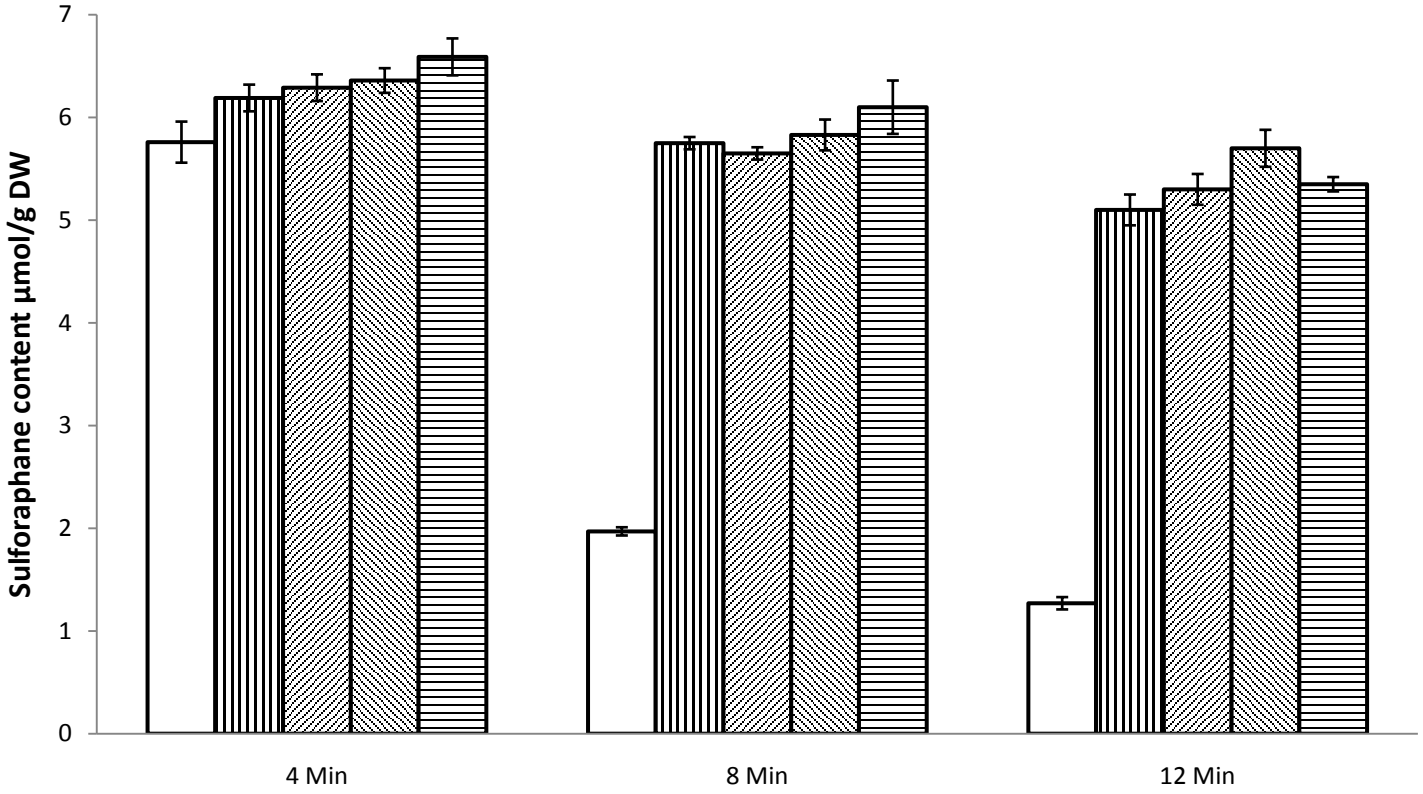

□ boiled broccoli, no mustard seeds; ▥ boiled broccoli with unprocessed MS, 1% ▨ boiled broccoli with unprocessed MS, 2%; ▧ boiled broccoli with processed MS (2%) for 5 min at 90 °C; ▤ boiled broccoli with processed MS (2%) for 5 min at 100 °C

Conclusions

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- In raw broccoli the predominant hydrolysis product of glucoraphanin is sulforaphane nitrile (more than 85%), due to the activity of epithiospecifier protein. However, mild cooking increases the conversion of glucoraphanin to sulforaphane, due to the inactivation of epithiospecifier protein while myrosinase is still partly active

- Domestic cooking leads to myrosinase activation, while glucoraphanin is still stable.

- Adding raw or slightly cooked **mustard seeds** to fully cooked broccoli provides a natural source of myrosinase enzyme needed to convert glucoraphanin to sulforaphane. This **guarantees** the conversion of all glucoraphanin to anticarcinogen sulforaphane, but not to sulforaphane nitrile which does not have antiarcinogenic properties.

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Sensory and consumer study

Gender	F/M (73.6%/26.4%)
Age	18-30 years (43.3%) 31-45 years (40.3%) 45-65 years (19.4%)
Broccoli consumption	Less than once a month (9.7%) More than once a month (45.8%) More than once a week (44.4%)
Cooking methods used for broccoli (consumers selected all that applied)	Steamed (36.1%) Boiled (62.5%) Microwaved (26.4%) Stir-fried (55.6%) Grilled (4.2%) Raw (12.5%)

Consumer liking of six broccoli samples prepared by different methods

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Sample	Overall liking	Liking of appearance	Liking of taste	Liking of texture
[1]SoVi,0%MS	6.2[b]±1.8	6.1[b]±1.8	6.4[a]±1.8	6.2[ab]±2.0
[2]SoVi,1%MS[7]	5.0[c]±2.2	5.2[c]±1.9	4.8[b]±2.4	5.8[b]±1.9
[3]SoVi,2%PMS	5.3[c]±2.0	5.4[bc]±2.1	5.4[b]±2.2	5.8[b]1.8
[5]NoBo,0%MS	7.1[a]±1.2	7.3[a]±1.2	6.9[a]±1.4	6.7[a]±1.5
[6]NoBo,1%MS	5.1[c]±2.1	6.0[bc]±1.8	5.0[b]±2.2	6.1[ab]±1.8
Direct comparison study (overall liking)				
SoVi,0%MS	6.3 [a] ±1.7			
[4]MilCo,0%MS	4.8 [b] ±2.3			

[1]: Sous vide cooking (100 ºC, 12 min, 0%MS); [2]: Sous vide cooking (100 ºC, 12 min, 1%MS) ; [3]: Sous vide cooking (100 ºC, 12 min, 2%processed MS); [4]: Mildly cooked, sous vide (70 ºC, 12 min, 0%MS); [5]: Normal boiling (100 ºC, 7 min, 0%MS); [6]: Normal boiling (100 ºC, 7 min, 1%MS); MS: Mustard seeds; different superscripts in the same column indicate significantly different means ($P<0.05$). Data are mean ± SD, $n=72$.

Liking of five clusters of consumers obtained from hierarchical cluster analysis

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Cluster	[1]SoVi 0%MS	[2]SoVi 1%MS[7]	[3]SoVi 2%PMS	[4]MilCo 0%MS	[5]NoBo 0%MS	[6]NoBo 1%MS
1(13.9%)	$6.8^{a}\pm1.3$	$3.9^{b}\pm1.3$	$3.0^{b}\pm1.2$	$6.3^{a}\pm2.0$	$7.2^{a}\pm1.1$	$3.8^{b}\pm1.0$
2(31.9%)	$6.1a^{b}\pm1.1$	$7.0^{a}\pm1.9$	$5.5^{b}\pm2.0$	$3.6^{c}\pm1.5$	$6.8^{ab}\pm1.9$	$6.7^{ab}\pm1.4$
3(19.4%)	$5.1b^{c}\pm1.7$	$3.6^{c}\pm1.6$	$6.6a^{b}\pm2.4$	$6.8^{ab}\pm1.0$	$7.0^{a}\pm1.6$	$3.4^{c}\pm0.8$
4(6.9%)	$6.0^{b}\pm0.5$	$6.6a^{b}\pm0.4$	$6.8a^{b}\pm0.7$	$7.8^{a}\pm1.7$	$7.0^{ab}\pm0.8$	$7.6^{ab}\pm0.7$
5(27.7%)	$7.0^{a}\pm1.9$	$3.8b^{c}\pm2.1$	$5.1^{b}\pm0.9$	$3.1^{c}\pm1.8$	$7.6^{a}\pm1.4$	$4.5^{bc}\pm1.2$
Overall liking	6.2	5.1	5.3	4.8	7.1	5.1

Conclusions

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- Isothiocyanate formation in broccoli could be increased by employing low intensity cooking conditions which leaves myrosinase in its active form, or by the addition of a natural source of myrosinase to fully cooked broccoli.
- Mild cooking option was not acceptable to consumers.
- Addition of mustard seed powder as an active source of myrosinase, significantly changed sensory attributes of broccoli samples and affected consumer liking.
- Despite the significant increase in pungency and burning sensation in samples with added mustard seeds, a considerable number of consumers (32%) liked it
- More work should be done to assess the effect of adding other *Brassica* condiments (e.g. rocket, watercress and horseradish) on the sensory attributes and consumer acceptance of cooked *Brassica* vegetables. In addition, isothiocyanate bioavailability should be assessed after adding those condiments to cooked *Brassica* vegetables.

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Aknowledments

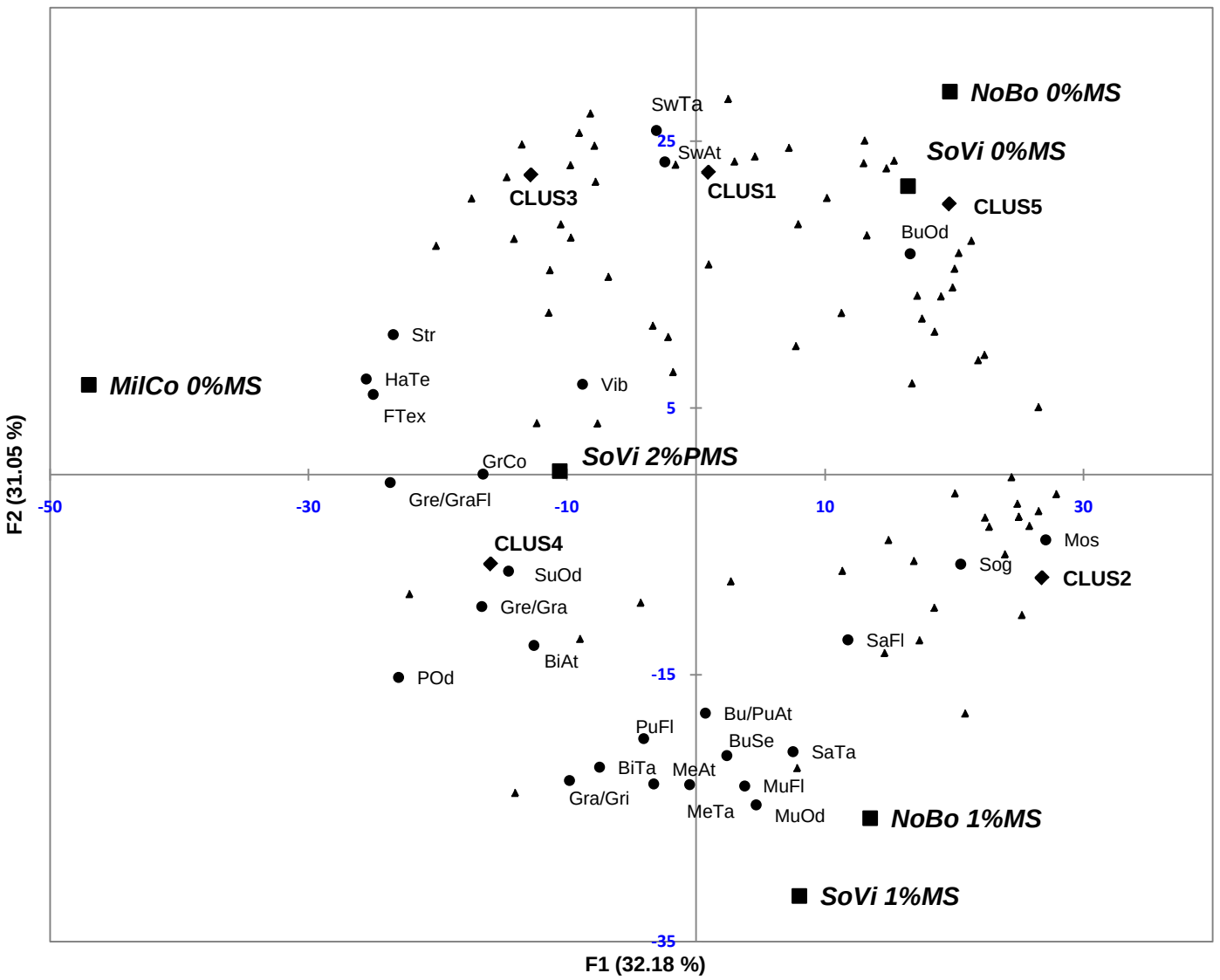
Prof. Keshavan Niranjan

Dr. Lisa Methven

Please know how to eat me!
Mr Broccoli..

Thank you for your patience, questions

Introduction1



The change in sulforaphane and sulforaphane quantities during processing

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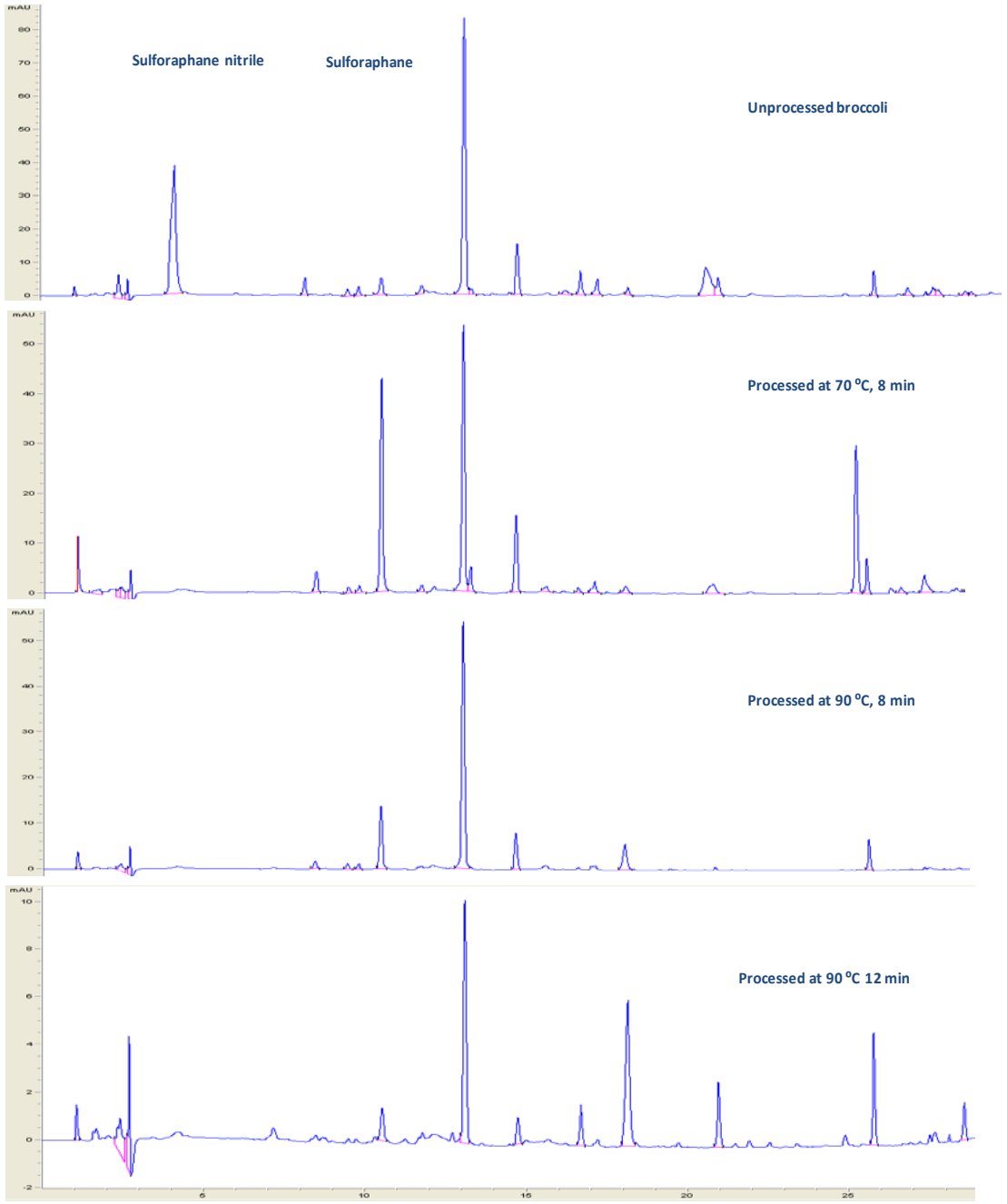

Sensory characterisation

	[1]SoVi 0%MS	[2]SoVi 1%MS	[3]SoVi 2%PMS	[4]MilCo 0%MS	[5]NoBo 0%MS	[6]NoBo 1%MS	Significance (p value)
Green Colour (Florette)	67.6 ab	65.3 ab	74.2 a	68.9 ab	58.7 b	60.4 ab	0.02
Evenness of green colour	52.6 a	55.7 a	62.5 a	61.8 a	55.3 a	55.0 a	0.38
Yellow (florets)	23.2 a	17.8 a	16.3 a	12.1 a	18.4 a	19.1 a	0.54
Vibrancy	43.0 b	42.7 b	48.4 ab	62.3 a	61.5 a	57.7 ab	<.01
Firmness of texture	41.8 bc	35.7 c	49.4 b	89.4 a	49.6 b	50.1 b	<.0001
Soggy	31.3 a	35.8 a	23.1 a	5.8 b	21.2 ab	20.0 ab	<.0001
Boiled green vegetables odour	57.0 a	52.0 a	47.3 a	46.7 a	44.9 a	43.4 a	0.37
Sulfur odour	12.0 a	13.4 a	10.2 a	13.0 a	7.3 a	12.0 a	0.08
Pungent odour	12.1 ab	19.2 ab	15.2 ab	21.2 a	8.2 b	15.1 ab	0.01
Mustard odour	0.3 b	18.1 a	11.0 a	0.0 b	0.0 b	9.7 ab	<.0001
Earthy odour	8.0 a	11.4 a	9.5 a	11.3 a	8.7 a	9.9 a	0.88
Green/grassy	34.3 ab	32.8 ab	29.6 ab	40.4 a	25.7 b	29.7 ab	0.07
Sweet odour	12.7 a	9.8 a	12.0 a	16.1 a	13.7 a	11.8 a	0.37
Savoury odour	8.5 a	12.9 a	10.1 a	9.7 a	7.8 a	10.8 a	0.38
Burnt odour	3.0 a	0.6 a	0.8 a	0.3 a	1.6 a	0.7 a	0.18
Bitter taste	14.2 ab	22.6 ab	26.5 a	18.9 ab	10.5 b	23.0 ab	0.03
Sweet taste	18.1 a	8.2 b	9.7 ab	17.2 ab	18.7 a	9.7 ab	<.01
Umami taste	10.3 ab	12.9 a	11.2 ab	10.3 ab	7.3 b	10.2 ab	0.07
Sour taste	3.3 a	4.7 a	4.0 a	2.2 a	2.0 a	3.1 a	0.32
Salty taste	3.7 ab	8.1 a	5.1 ab	2.6 b	2.4 b	4.5 ab	0.02
Metallic taste	7.9 ab	11.8 a	12.5 a	7.0 ab	3.7 b	12.5 a	<.01
Boiled green vegetables flavour	48.6 a	43.0 a	42.2 a	37.7 a	39.6 a	37.2 a	0.55
Sulfur flavour	10.0 a	14.3 a	13.1 a	6.3 a	6.2 a	9.8 a	0.08
Pungent flavour	9.6 cd	27.9 ab	37.1 a	12.6 bcd	4.8 d	25.1 abc	<.0001
Mustard flavour	0.3 b	41.8 a	43.2 a	0.0 b	0.0 b	36.7 a	<.0001
Earthy flavour	8.6 a	11.2 a	14.6 a	9.7 a	8.2 a	9.1 a	0.41
Green/grassy flavour	30.6 ab	29.3 ab	27.2 b	40.6 a	26.4 b	25.7 b	0.01
Savoury flavour	11.3 ab	14.8 a	12.9 ab	6.7 b	7.5 b	10.8 ab	0.01
Burnt flavour	1.3 a	1.2 a	1.5 a	0.0 a	0.0 a	0.7 a	0.62
Stalk flavour	11.4 a	13.7 a	8.7 a	18.0 a	11.1 a	14.3 a	0.36
Moist	49.8 a	49.2 a	46.9 a	28.4 b	48.3 a	51.6 a	<.0001
Hard texture	32.6 b	25.7 b	35.6 b	80.6 a	37.6 b	34.2 b	<.0001
Grainy/gritty	0.4 b	5.3 ab	9.0 a	3.9 ab	1.0 ab	7.2 ab	0.02
Burning sensation	0.0 c	41.6 ab	51.4 a	0.0 c	0.3 c	35.2 b	<.0001
Stringy	7.4 b	4.3 b	4.7 b	22.9 a	8.1 b	3.2 b	<.01
Burn/pungncy aftertaste	2.6 c	30.3 b	46.5 a	1.9 c	1.5 c	24.7 b	<.0001
Bitter aftertaste	7.4 c	18.7 a	17.4 ab	12.3 abc	5.8 c	8.2 bc	<.01
Sweet aftertaste	9.6 a	3.8 a	5.1 a	7.5 a	8.4 a	3.2 a	0.03
Savoury aftertaste	8.5 a	13.4 a	10.2 a	7.5 a	6.0 a	7.7 a	0.07
Earthy aftertaste	6.0 a	7.3 a	7.7 a	11.1 a	4.7 a	7.7 a	0.34
Metallic aftertaste	4.7c	13.5a	12.3ab	6.1bc	1.2c	10.2abc	<.0001