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FREE THIOCYANATE ION: A HYDROLYSIS PRODUCT OF GLUCOSINOLATES FROM RAPE AND MUSTARD SEED MEAL

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Glucosinolates of Brassica species hydrolyze under controlled conditions of neutral pH and the presence of a myrosinase isolate from yellow mustard (Brassica hirta) to yield, in addition to glucose and sulphate, isothiocyanates. Most of these isothiocyanates are stable in aqueous solution (1). Some may be assayed for by gas-liquid chromatography (2), by titration following steam distillation (3) or by spectrophotometry after conversion to thiourea derivatives (4, 5), while others may be assayed either by gas-liquid chromatography (6) or spectrophotometry (2, 4, 5, 7) after cyclization to oxazolidinethiones. A few of the isothiocyanates, however, are not stable in aqueous solution and readily break down, particularly at alkaline pH, to yield free thiocyanate ion (1). These cannot be assayed by gas-liquid chromatography, by steam distillation and titration or by spectrophotometry of thiourea derivatives. Although the glucosinolates commonly associated with the seed of rapeseed (B. napus and B. campestris) and brown or Oriental mustard (B. juncea) (8) are thought to yield only isothiocyanates which are stable in aqueous solution (1), recently it was shown that autolysis with endogenous myrosinase or hydrolysis with a yellow mustard myrosinase isolate of rapeseed meal produced free thiocyanate ion (9).

In order to determine if the precursor of this thiocyanate ion could be a glucosinolate, the amounts of glucose, sulphate, isothiocyanates, (including oxazolidinethiones) and thiocyanate ion released by aqueous extracts of meal from heated seed of B. napus cv. Tower following hydrolysis with a yellow mustard myrosinase isolate were compared. Glucose was determined enzymatically by the hexokinase glucose-6-phosphate dehydrogenase method, sulphate by titration with barium chloride using thorin indicator, isothiocyanates by reaction with ammonia to form thioureas or cyclization to oxazolidinethiones, and thiocyanate ion by reaction with ferric nitrate (10). The aqueous extracts of the Tower seed meal had relatively low amounts of thiocyanate ion, but upon incubation with myrosinase yielded appreciable amounts (Table 1).

TABLE 1

CONTENT OF GLUCOSINOLATE BREAKDOWN PRODUCTS IN AQUEOUS EXTRACTS OF OIL-FREE TOWER MEAL INCUBATED WITH AND WITHOUT MYROSINASE

Enzyme treatment	Glucose	Sulphate	Isothiocyanate	Thiocyanate ion
		μmoles/g oil-free meal*		
+myrosinase	25.0±0.5	59.7±0.8	11.3±0.3	14.6±0.1
-myrosinase	2.6±0.5	37.9±0.7	-	1.2±0.1
Difference	22.4	21.8	11.3	13.4

*Mean and standard error of the mean for duplicate analysis on three extracts.

Stoichiometric agreement between the glucose, sulphate and the combined amounts of isothiocyanates and thiocyanate ion, as determined from the difference between incubation of the extracts in the presence or absence of myrosinase, indicated that in addition to isothiocyanates thiocyanate ion is a product of glucosinolate hydrolysis.

Meals of Canadian commercial cultivars and strains of both B. napus and B. campestris rapeseed and B. juncea mustard seed were analyzed for thiocyanate ion following incubation with a yellow mustard myrosinase isolate. Hydrolysates were treated with sodium hydroxide to ensure breakdown of any unstable isothiocyanates (10). Meals from both species of rapeseed released comparable amounts of thiocyanate ion (Table 2). Mustard seed meals released less.

TABLE 2

YIELD OF GLUCOSINOLATE BREAKDOWN PRODUCTS IN BRASSICA CULTIVARS AND STRAINS

Species & cultivar	Volatile isothiocyanates	Oxazolidine-thione	Thiocyanate ion	Total glucosinolate
		µmoles/g oil-free meal*		
B. napus				
Nugget	45.1 b	71.1 d	13.1 a	129.3
Turret	46.4 ab	78.8 c	12.4 b	137.6 c
Midas	52.6 ab	85.8 b	11.7 c	150.1 ab
Golden	53,7 a	78.6 c	11.5 cd	143,8 b
Tanka	51,5 ab	91.0 a	11.4 cd	153.9 a
Tower	7.5 d	10.7 e	11.4 cd	29.6 e
Target	46.2 ab	78.2 c	11.0 de	135.4 c
Oro	36.7 c	77.1 c	10.8 e	124.6 d
SZN71-1788	4.9 d	8.7 e	10.1 f	23.7 ef
Zephyr	44.9 b	75.7 c	10.1 f	130.7 cd
Bronowski	6.0 d	5.2 f	8.6 g	19.8 f
B. campestris				
Span	52.8 d	23.2 b	11.8 a	87.8 c
Arlo	54.8 cd	21.1 c	11.6 ab	87.5 c
Torch	55.3 bcd	21.7 bc	11.3 ab	88.3 c
Echo	61.9 bc	25.6 a	11.2 ab	98.7 b
Polar	63.2 b	23.0 b	11.2 b	97.4 b
R500	121.9 a	7.9 d	9.4 c	139.2 a
CZY3-1820	11.1 e	3.3 e	8.4 d	22.8 d
B. juncea				
Commercial brown	116.9 c	-	6.9 a	123.8 c
Stoke	145.9 a	-	6.2 b	152.1 a
Ekla	124.9 b	-	5.6 c	130.5 bc
Leth.22A	131.2 b	-	5.4 c	136.6 b

*Mean of duplicate determinations on seed from two replicate plots. Duncan's multiple range test applied to each species separately ($P < .05$).

It is of interest that meals from low glucosinolate and high glucosinolate seed released similar amounts of thiocyanate ion. This indicates that breeding for reduced glucosinolate content in rapeseed has not reduced the precursor of the thiocyanate ion. Low amount of thiocyanate ion released by CZY3-1820, a sister line to the recently licensed yellow-seeded rapeseed cultivar Candle, may reflect its genetic background. The low glucosinolate characteristic was introduced into this line by interspecific crosses between B. napus, B. campestris and B. juncea.

Estimates of the total glucosinolate content of the seed meals were obtained after incubation with a yellow mustard myrosinase isolate by determining the stable isothiocyanates as their thiourea derivatives and oxazolidinethiones (10) and adding these values to the values obtained for thiocyanate ion. For low glucosinolate cultivars the amount of thiocyanate ion released almost equalled the isothiocyanates (Table 2). Current methods which indirectly determine the glucosinolate content of rapeseed by measuring for the isothiocyanates produced by myrosinase hydrolysis include only those isothiocyanates which are stable in aqueous solution whether the volatile isothiocyanates are determined by the argentimetric method involving steam distillation and titration (3), by gas-liquid chromatography (2) or conversion to thiourea derivatives (4, 5) and the non-volatile isothiocyanates following cyclization to oxazolidinethiones (2, 4, 5, 6, 7). If determination of the glucosinolate content of low glucosinolate seed is not to be grossly underestimated, then it would appear that these methods should be complemented with analysis for free thiocyanate ion. Discrepancy between the glucosinolate content of low glucosinolate seed as determined by glucose measurement and direct analysis of the glucosinolates as their trimethylsilyl derivatives (11) suggests that the latter methods (12, 13) should also be complemented by analysis for thiocyanate ion.

The identity of the thiocyanate ion precursor is not yet known. It is of interest that indolylglucosinolates whose isothiocyanates are unstable in aqueous solution and breakdown to free thiocyanate ion are known to be present in the vegetative parts of rapeseed (1), and although reported not to be present in the seed (1) are present in substantial amounts in the seed of the Cruciferae Woad (Isatis tinctoria) (14). Paper chromatography of aqueous extracts from heated seed of B. napus Tower in the solvent system 1-butanol-acetic acid-water (4:1:2 v/v) (14) has yielded, in addition to tryptophan Rf 0.59, two bands, compound 1 Rf 0.23 and compound 2 Rf 0.35 which appear to be indole in nature. Both react when sprayed with Ehrlich's and sodium nitrite (15) to give gray blue and blue colors respectively.

A solvent mixture consisting of methyl ethyl ketone-n-butanol-benzene-ammonium hydroxide-2-propanol (1:2:2:2:3 v/v) (MBBAP) was developed to separate compounds 1 and 2 on silica gel G by thin layer chromatography. Modification of a solvent mixture of Matsuo's (16) consisting of methyl ethyl ketone-ethanol-water-acetic acid (9:1:1:1 v/v) was used to separate compounds 1 and 2 from the known glucosinolates of rape and mustard seed. Both compounds 1 and 2 yielded thiocyanate ion (Rf 0.50 in MBBAP) when hydrolyzed at pH 7.0 with a yellow mustard myrosinase isolate. Based on the intensity of the reaction with Ehrlich's sodium nitrite there appears to be more of compound 1 than compound 2 in rapeseed. As the seedling develops, however, this rapidly reverses. On the basis of Rf and color reaction compound 2 may be 3-indolylmethyl glucosinolate (glucobrassicin).

From a biochemical viewpoint it is of interest that breeding for reduced glucosinolate content has not reduced the precursor for the thiocyanate ion. If the precursors are indolylglucosinolates, it would support the suggestion (1) that the metabolic block introduced through breeding for low glucosinolate is located in the amino acid chain elongation steps which are part of the pathway for glucosinolate synthesis from methionine and phenylalanine but are not required for synthesis of indolylglucosinolates from tryptophan (17).

Nutritional studies recently have emphasized the toxicity of nitriles formed upon autolysis of rapeseed (18). But for low glucosinolate seed the addition of nitrile to detoxified meal at a level corresponding to the amount found in autolyzed meal did not produce as great a growth depression as the autolyzed meal (18). However, the level of nitrile added was based on the content of known glucosinolates as determined by thiourea and oxazolidinethione analysis. Glucosinolates which hydrolyze to free thiocyanate ion may also autolyze to nitriles and contribute to anti-nutritional properties. Since plant breeding for low glucosinolate has not reduced the precursor of the thiocyanate ion it would appear that reduction of the glucosinolate content is less than previously thought. Considerably reduction in the glucosinolate content of rapeseed is therefore still possible and might eliminate any anti-nutritional properties.

REFERENCES

1. Josefsson, E., 1970. Swedish Seed Ass. Publ.
2. Youngs, C.G. and L.R. Wetter, 1967. J.Am.Oil Chem.Soc. 44: 551-554.
3. Wetter, L.R., 1955. Can.J.Biochem.Physiol. 33: 980-984.
4. Appelqvist, L-A. and E. Josefsson, 1967. J.Sci.Fd. Agric. 18: 510-519.
5. Wetter, L.R. and C.G. Youngs, 1976. J.Am.Oil Chem.Soc. 53: 162-164.
6. Daxenbickler, M.E. et al., 1970. Anal.Biochem. 38: 373-382.
7. Wetter, L.R., 1955. Can.J.Biochem.Physiol. 35:293-297.
8. Appelqvist, L-A., 1972. In Rapeseed; cultivation, composition, processing and utilization, p.149.
9. Srivastava, V.K. and D.C. Hill 1975. Can.J.Biochem. 53: 630-633.
10. McGregor, D.I., Can.J.Plant Sci. (in press).
11. Olsson, K. et al., 1976. Swedish J.Agric. Res. 6: 225-229.
12. Persson, S., 1974. In Proc. 4th Intern.Rapeseed Conf., Giessen, W.Germany, pp. 381-386.
13. Thies, W., 1976. Fette Seifen Anst. 78: 231-234.
14. Elliot, M.C. and B.B. Stowe, 1971. Plant Physiol. 47: 366-372.
15. Sprince, H., 1960. J.Chromatog. 3: 97-98.
16. Matsuo, M., 1970. J.Chromatog. 49: 323-324.
17. Underhill, E.W. et al, 1973. Biochem.Soc.Symp. 38: 303-326.
18. Josefsson, E., 1975. J.Sci.Fd.Agric. 26: 1299-1310.