Gamma-Irradiation to Inactivate Thioglucosidase of Crucifers[1]

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The crucifers contain glucosinolates which through enzymatic hydrolysis give rise to toxicants that limit the use of oil-free meal obtainable from this plant family. Seeds from three crucifers were used to test gamma irradiation to inactivate enzyme systems as a step toward detoxification. Seeds of *Crambe abyssinica* Hochst (crambe), ground seeds of *Sinapis alba* L. (mustard), and seeds of *Brassica napus* L. (rape) were subjected to gamma-irradiation (6.25, 12.5, 25.0 and 50.4 Mrad) to inactivate thioglucosidase and/or destroy glucosinolates. Samples of ground seeds, their oil-free meals, previously irradiated ground seeds and their oil-free meals were assayed for glucose, a product of enzymatic hydrolysis of glucosinolates present in the crucifer seeds. The 50.4 Mrad exposure inactivated thioglucosidase but did not destroy glucosinolates. The fatty acid contents of extracted oils were affected. The amino acid profile of defatted crambe protein meal was affected, while that of white mustard was not.

Glucosinolates in crucifers exemplify antiquality agents (1) that are often present in food and feed plants and are a major problem in the utilization of their seed protein. Glucosinolates are hydrolyzed by endogenous thioglucosidase (EC3.2.3.1) to a toxic mixture of sulphate, isothiocyanate, thiocyanate, organic nitriles, episulfides and sulphur (2,3). Treatments such as hot air, steam, boiling water and microwave (4-6) to inactivate the thioglucosidase, extract the glucosinolates and improve the feeding value of the protein from the crucifer crambe have been reported. These treatments did not change the fatty acids composition of the extracted oil and did not alter the amino acid profile of the meal protein.

Moist heat destroys the ability of enzymes to act on the glucosinolates, and removal of glucosinolates by water washing and filtering after heat treatment of oil-free meal has been reported (3,7). Except for palatability, ingestion of glucosinolates in the absence of thioglucosidase is reported as being "not particularly harmful" to beef cattle, but harmful to other animals, especially monogastric creatures (8,9). Procedures that have been used to inactivate thioglucosidase are generally energy intensive, and quality control is difficult. Depending upon the availability of waste radioisotope resources from nuclear industries, irradiation may offer a more cost effective and controlled alternative to inactivate thioglucosidase.

The use of gamma-irradiation in the disinfestation or disinfection of foods and in the storage of foods has been studied (10). However, the use of high doses of gamma-irradiation to inactivate antiquality factors in potential foodstuffs is preliminary (11,12). Isothiocyanate and oxazolidinethione reduction in rapeseed meal has been reported from 50.0 Mrad gamma-irradiation (13). The effects of gamma irradiation upon certain vegetable oils and their constituents, triglycerides and free fatty acids, has been examined in detail and excellent reports presented (14-21). However, no reports on the effects of high doses of gamma-irradiation on enzyme activity or integrity of their substrates in intact or gound crucifer seeds and subsequent examination of extracted oils and amino acid profiles of oil-free protein meals were found in the literature. The research reported here examined effects of gamma-irradiation at high dosages on thioglucosidase activity and glucosinolate integrity in the protein meal of crucifers. Effects on oil and protein integrity of ground seeds and oil-free meal were not the primary objectives of the research, but were examined as a part of the work.

MATERIALS AND METHODS

Seeds of (Meyer) crambe, boiled seeds [$H_2O\Delta$(180 sec)] of (Meyer) crambe, ground seeds of white mustard and seeds of (Regent) rape, which is low in erucic acid (22), were treated with gamma-irradiation (6.25, 12.5, 25.0, 50.4 Mrad) at Sandia Laboratories, Albuquerque, New Mexico. All samples were $\geq$ 20 g at 3-4% moisture.

Irradiation treated and untreated samples of ground seeds were checked for thioglucosidase (EC.3.2.3.1) activity by testing for glucose, formed from glucosinolate hydrolysis, with commercially available Tes-Tape (Eli Lilly Co., Indianapolis, Indiana) (2) and as used in commercial-scale work on processing crambe (8). The presence of glucose in moistened ground-seed samples serves as a test of thioglucosidase activity (8).

To determine effects of irradiation on seed oil and amino acid profiles of oil-free protein meals, comparisons of these seed components were made between untreated, irradiated and water-heated (immersed in boiling water for 180 sec) crambe seeds and untreated and irradiated rape and ground white mustard seeds. The amount of oil extracted from all materials was determined according to AOCS method BA 3-38. The fatty acid composition of extracted oils was analyzed by gas liquid chromatography (GLC), as methyl esters, at the Northern Regional Research Center, USDA, Peoria, Illinois and in our laboratory with a Varian Model 3700 gas chromatograph equipped with a G.P. 10% Sp 2330 on 100/120 chromosorb WAW 6′ × 1/8″ SS column. Three chromatograms per oil sample were obtained.

For amino acid analyses ground defatted samples (25 mg) of irradiated and non-irradiated crambe and white mustard seeds were dispersed in 100 ml of 6 N HCl and hydrolyzed with a reflux condenser at 110 C for 24 hr. Oxygen was removed from the condenser initially by addition of a pellet of solid CO_2. Hydrolysates were evaporated to dryness on a rotary evaporator at 50 to 60 C, dissolved in 10 ml citrate buffer pH 2.2 and filtered. Analyses were made with a Durham 400 Amino Acid analyzer in the Chemistry Department at New Mexico State University.

[1]This is Scientific Paper 211 of the Agricultural Experiment Station, New Mexico State University, Las Cruces, NM 88003.

To establish the presence of glucosinolates in irradiated and hot water-treated ground seed samples and defatted meal showing no thioglucosidase activity, exogenous myrosinase, prepared from white mustard (*Sinapis alba* L.) seed (23), was added to treated sample preparations and samples tested for glucose as before. A positive test for glucose indicates presence of glucosinolates, but no activity of endogenous thioglucosidase (8).

RESULTS AND DISCUSSION

Tests for glucose as an indication of thioglucosidase activity after irradiation treatments are presented in Table 1. Irradiation at 50.4 Mrad apparently inactivated thioglucosidase in these crucifers. All samples after hot water treatment showed no thioglucosidase activity by the glucose test.

Exogenous myrosinase was added to all samples to detect the presence of glucosinolate in those showing inactivation of thioglucosidase by the glucose test. As can be seen in Table 1, a positive test for glucose was obtained in those samples showing inactivation of the enzyme after $H_2O\Delta$(180 sec) and 50.4 Mrad treatments indicating no major modification of glucosinolates.

Since an important crucifer seed constituent is oil, knowledge concerning possible effects of irradiation on oil extraction rates and fatty acid composition of extracted oils is important. Therefore, Table 1 shows thioglucosidase inactivation, glucosinolate integrity and extractable oil contents of irradiated, hot water-heated and untreated samples. Enzyme inactivation was achieved without any apparent negative effects on oil extraction when compared to untreated samples. In fact, statistical analysis of data (24) indicates a significant increase in oil recovery from materials treated at the higher irradiation dosage levels within seed lots (Table 1). Oil percentages are presented on a dry weight basis; therefore, the trend toward increased recovery is not expected to be a result of moisture losses from irradiation. It may be irradiation caused the development of constituents that became soluble in the solvent used for extraction and this added weight to the oil sample but were not detected by the GLC column used. More work is indicated to determine the exact nature of the increased recovery measured.

Differences in total oil content between boiled crambe seeds (grown in 1981) and non-boiled crambe seeds (grown in 1982) are believed due to different seed lots used. Average total oil yields are within the range of oil contents of (Meyer) crambe grown in different years in New Mexico.

Table 2 shows that oil-free meals from the $H_2O\Delta$(180 sec) and 50.4 Mrad-treated materials tested negative for glucose and positive when exogenous myrosinase was added.

The GLC results of fatty acid composition of untreated, hot water- and irradiation-treated materials show a range in erucic acid (C22:1) content from 54.0% in untreated crambe seeds to 60.0% in boiled crambe seeds treated at the 50.4 Mrad level (Table 3). Differences in erucic acid content of this magnitude are often found among a group of oil samples of (Meyer) crambe (25). However, statistical analyses of these data and that obtained from the other crucifers (24) indicates a significant consistent shift to a higher percentage of erucic acid in the samples when irradiation dosage was increased. Also, there is a significant shift to less

TABLE 1

Glucose Tests and Oil Content of Ground Seed Samples from Gamma-Irradiated Material

Material	Treatment	Glucose test: before myrosinase added	Glucose test: after myrosinase added	% Oil
Crambe seed[a]	check	+	+	28.8
	6.25[b]	+	+	28.7
	12.5	+	+	29.6
	25.0	+	+	29.6
	50.4	−	+	30.0
Boiled crambe seed[c]	check	−	+	36.0
	6.25	−	+	37.0
	12.5	−	+	40.0
	25.0	−	+	41.0
	50.4	−	+	40.5
Ground Mustard seed[a]	check	+	+	27.4
	6.25	+	+	28.2
	12.5	+	+	29.2
	25.0	+	+	29.3
	50.4	−	+	29.3
Rapeseed[a]	check	+	+	43.2
	6.25	+	+	44.5
	12.5	+	+	46.5
	25.0	+	+	46.5
	50.4	−	+	47.5

[a]Grown in 1981.
[b]Mrad.
[c]Grown in 1982.

TABLE 2

Glucose Test of Oil-Free Meal Samples from Crucifer Materials After $H_2O\Delta$(180) and 50.4 Mrad Gamma-Irradiation

Material	Treatment	Glucose test: before myrosinase added	Glucose test: after myrosinase added
Crambe seeds[a]	50.4[b]	−	+
	check	+	+
Boiled crambe[c]	50.4	−	+
	check	−	+
Ground Mustard seed[a]	50.4	−	+
	check	+	+
Rapeseed[a]	50.4	−	+
	check	+	+

[a]Grown in 1981.
[b]Mrad.
[c]Grown in 1982.

linoleic (C18:2) and linolenic (C18:3) fatty acids and an increase in oleic (C18:1) acid with increased exposure to irradiation. The same shifts may be observed in the fatty acid composition of white mustard oil samples and in samples from rapeseed. The plotted slopes of these value changes were estimated by regression, and r^2 values ranging from .52 to .99 were calculated. Oil from the rape cultivar (Regent) contains very little erucic (C22:1) acid (22). The relative amounts of other fatty acids appear not to differ as greatly from treatments, but we conclude that seed treatments used to inactivate thioglucosidase did affect the balance of the most prevalent fatty acids in extracted oils. This might be expected based on reports on the effects of gamma-irradiation treatments on vegetable oils, triglycerides and free fatty acids (14-19). Nawar (14) reported no significant changes in complete fatty acid composition of vegetable fats after irradiation (6 Mrads). However, he did report that irradiation resulted in production of hydrocarbons, aldehydes, methyl esters and ethyl esters. In other reports by Nawar et al. (15,17,20,21) it is stated that "the radiolytic breakdown of triglycerides yields a series of saturated and unsaturated hydrocarbons, the type and quantity of which depend on the glyceride fatty acid composition. Of the hydrocarbons produced from each fatty acid, two were formed in relatively large quantities." It was observed that "the production of the 'major' hydrocarbons increased linearly with an increase in radiation temperature or dose." Meidani et al. (16) reported that some larger recombination products expected from irradiation (6 Mrads) of tricaproin were not observed. Those compounds were considered difficult to detect using gas chromatography. At irradiation levels used in the work reported here we suspect that products were formed that likewise were not detected during chromatography. We do not propose that oleic ($C_{18:1}$) or erucic ($C_{22:1}$) acids were increased from treatments, in the absolute sense, but that perhaps some of the linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids may have dimerized or further polymerized and were no longer measured as such by GLC with the column used. Although complete analysis of radiolytic products of triglycerides was not the major objective of this work, we feel the results suggest the need for the use of an internal standard (26) in further GLC analyses of fatty acid changes as a result of gamma-irradiation of crucifer seeds and oils. The use of an internal standard which might have been used in this work should allow an absolute measure of any changes in individual fatty acid constituents if they occur. Since irradiation may cause modification in major fatty acid content or create undesirable radiolytic products in exposed oils, its use to inactivate enzymes that can hydrolyze glucosinolate

TABLE 3

Fatty Acid Composition of Extracted Crucifer Oils[a] After Gamma-Irradiation Treatments of Seed

Material	Treatment	Myristic (C14:0)	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C22:0)	Erucic (C22:1)
Crambe seed[b]	Untreated	0.10	2.0	0.4	1.0	17.0	12.0[c]	11.0[c]	3.0	54.0[c]
	6.25[c]	0.06	2.0	0.3	1.0	17.0	12.0	10.0	3.0	54.0
	12.5	0.06	2.0	0.3	1.0	18.0	11.0	10.0	4.0	54.0
	25.0	0.06	2.0	0.3	1.0	18.0	11.0	10.0	3.0	55.0
	50.4	0.30	3.0	0.4	1.0	18.0	10.0[c]	9.0[c]	2.0	57.0[c]
Boiled crambe seed[d]	Untreated	0.10	2.0	0.3	1.0	17.0	12.0	10.0	2.0	56.0
	6.25	0.10	2.0	0.3	1.0	17.0	11.0	10.0	2.0	57.0
	12.5	0.10	2.0	0.2	1.0	17.0	11.0	9.0	2.0	58.0
	25.0	0.20	3.0	0.3	1.0	18.0	10.0	9.0	2.0	60.0
	50.4	0.30	2.9	0.3	1.0	18.0	9.0	8.0	1.0	60.0
Mustard seed[b]	Untreated	0.04	3.0	0.2	1.0	24.3	11.0	21.0	0.3	38.7
	6.25	0.04	3.0	0.2	1.0	25.7	11.0	20.3	0.2	38.0
	12.5	0.04	3.0	0.2	1.0	27.5	8.5	17.0	0.2	42.0
	25.0	0.04	3.0	0.3	1.0	27.0	9.0	18.0	0.3	42.0
	50.4	0.04	4.0	0.3	1.0	28.0	8.0	16.0	0.3	42.3
Rapeseed[b]	Untreated	0.04	4.7	0.2	2.0	60.7	21.3	10.3	0.1	0.3
	6.25	0.03	4.0	0.2	2.0	61.0	21.0	11.0	0.3	0.01
	12.5	0.03	4.0	0.3	2.0	61.3	21.0	10.0	0.3	0.06
	25.0	0.03	4.0	0.3	2.0	62.0	21.0	10.0	0.3	0.01
	50.4	0.03	4.07	0.3	2.0	63.7	20.0	9.0	0.3	0.02

[a]Percentages are mean values from three chromatographs per oil sample.
[b]Grown in 1981.
[c]Mrad.
[d]Grown in 1982.
Decrease of 4.0% in 18.2 and 18.3:∴ 100%−4/5 = 96% of acids remain in irradiated sample. ∴ starting 22:1 should be increased by 54.0% ÷ 0.96 = 57%. Similar calculations for other samples also fit this destruction rationale.

into feed toxicants may require irradiation of oil-free meals rather than seeds or gound seeds to avoid exposure of oil components to the effects of gamma-irradiation. For crambe or rapeseed, however, irradiation probably increases the stability of the oil by destroying the triene components that lead to oxidative instability.

The effects of using gamma-irradiation to inactivate enzyme systems that lead to toxic feed constituents of oil-free meals and fatty acid integrity of oils after extraction are of interest. However, it is important to have information about effects of irradiation on the amino acid composition of oil-free meals of crucifers if they are to be used as food or feed. Upon first observation of the amino acid composition of untreated, hot water-heated, and irradiated meals it might seem that irradiation up to 50.4 Mrad caused no major changes in the amino acid composition of the crucifer seed proteins (Tables 4 and 5, respectively). However, after regression analysis there is statistical evidence (.05 level) that the eight amino acids (aspartic, cystine, histidine, leucine, lysine, methionine, phenylalanine and tyrosine) were reduced, but only in defatted crambe meal. The r^2 values ranged from .81 to .96. There is no statistical evidence of any effect upon the amino acid composition of mustard seed meal. From regression analysis, r^2 values were .27 or less. There was a reduction in percent total nitrogen recovered and percent nitrogen as amino acids in crambe meal samples after treatment to 50.4 Mrad. The r^2 values were .94 and .92, respectively. There was no effect of treatments on percent protein. No other samples showed such a trend. Though amino acid composition of the protein may or may not change, fish products treated with 5 Mrad to 50 Mrad (12), for example, showed no accumulation of histamine; the protein itself might be affected. It has been shown (27) that changes in flavor, odor, color and texture of irradiated foods do occur. So it is important that any chemical changes produced by irradiation do not reduce nutrient value or produce carcinogenic compounds. Some of the first long term feeding trials with rats fed irradiated butterfat (27) and beef (28) showed no evidence of carcinogenicity (27), and it is reported that animals performed very well over successive generations when fed irradiated beef hamburger (28). Yet many experiments have shown that certain essential nutrients are destoyed to varying degrees when foodstuffs are subjected to sterilizing doses of irradiation (27). We feel there is a need to evaluate feed or food products that have been subjected to levels of radiation as used in this work. Though preliminary data reported have suggested there are practically no qualitative differences between radiolytic compounds obtained at low and high doses (29), quantitative differences do occur at a 25 Mrad exposure versus 6 Mrad (30).

In summary, enzyme inactivation by either hot water or irradiation at 50.4 Mrad appears to have no adverse effects on the percent recovery of oils but some effects on amino acid composition of crambe seed

TABLE 4

Amino Acid Composition of the Protein in Oil-Free Crambe Meal Before and After Gamma-Irradiation of Seeds

		Irradiation treatments[a]			
Amino acid	Untreated[a]	50.4	25.0	12.5	6.25[b]
Lysine	5.15	4.79	4.92	5.08	5.29
Methionine	1.58	1.35	1.44	1.56	1.61
Isoleucine	3.71	3.46	3.63	3.73	3.93
Leucine	6.31	5.90	6.18	6.24	6.51
Phenylalanine	3.86	3.62	3.67	3.81	3.90
Arginine	6.83	6.37	6.83	6.49	6.85
Tyrosine	2.86	2.52	2.71	2.73	2.86
Threonine	4.34	4.00	4.18	4.38	4.09
Valine	3.89	4.26	3.73	3.94	4.92
Histidine	2.40	2.19	2.32	2.39	2.40
Glycine	5.34	4.87	5.15	4.99	5.10
Proline	5.73	5.87	6.03	5.97	6.55
Alanine	4.19	3.88	4.10	3.95	3.98
Aspartic acid	6.59	5.71	6.26	6.19	6.21
Glutamic acid	17.16	15.33	17.04	16.86	16.72
Serine	3.95	3.75	3.81	3.97	3.78
Cystine	1.85	1.27	1.36	1.74	1.73
% Nitrogen as amino acids	74.93	69.37	73.11	73.14	75.46
% Total nitrogen recovered	86.86	80.73	83.88	85.41	87.69
%Protein (N × 6.25)	45.68	45.37	47.34	47.37	47.43

[a]g/16gN.
[b]Mrad.

TABLE 5

Amino Acid Composition of the Protein in Oil-Free White Mustard Meal Before and After Gamma-Irradiation of Ground Seeds

		Irradiation treatments[a]			
Amino acid	Untreated[a]	50.4	25.0	12.5	6.25[b]
Lysine	5.70	5.52	5.48	5.06	5.98
Methionine	1.60	1.41	0.95	1.61	1.73
Isoleucine	3.70	3.73	3.69	3.59	3.46
Leucine	6.80	6.83	6.76	6.55	6.92
Phenylalanine	3.73	3.94	3.76	3.50	4.00
Arginine	6.54	6.07	5.75	5.72	6.29
Tyrosine	3.15	3.01	3.44	2.92	3.32
Threonine	4.14	4.18	4.24	4.29	4.25
Valine	4.18	5.05	4.86	4.11	5.15
Histidine	2.83	2.78	2.50	2.38	2.98
Glycine	5.50	5.46	5.42	5.69	5.60
Proline	6.11	6.90	7.00	7.42	6.04
Alanine	4.12	4.17	4.15	4.38	4.13
Aspartic acid	7.74	7.66	7.44	7.88	7.49
Glutamic acid	17.29	16.51	16.06	18.49	17.17
Serine	4.07	4.11	4.07	4.29	4.24
Cystine	1.22	1.05	1.34	0.76	1.04
% Nitrogen as amino acids	77.11	76.48	74.80	75.65	78.06
% Total nitrogen recovered	88.90	88.92	87.21	87.00	90.75
% Protein (N × 6.25)	44.18	44.56	43.62	46.75	44.50

[a]g/16gN.
[b]Mrad.

proteins. Therefore, irradiation may prove useful even if plant breeding or genetic engineering is successful in reducing glucosinolate synthesis in crucifers. Toxicity due to hydrolysis of glucosinolates in potential feeds and foods is so severe that effective extraction processes to remove toxicants could serve as a safeguard against seed mixtures or seeds of potential glucosinolate-containing crucifers that may get into the processing chain. If irradiation of crucifer seeds and oil-free meals does carry the risk of adversely affecting recovered protein meals, these meals may find use as a source of extractable amino acids, or after proper evaluation as a source of protein concentrate (32).

ACKNOWLEDGMENTS

The authors appreciate the technical assistance of Jim Pierce, Sandia National Labs, Albuquerque, New Mexico, with the irradiation process, and of J.H. Hageman, Department of Chemistry, New Mexico State University, with amino acid analyses. Partial funding was supplied by the U.S. Department of Energy, Albuquerque Operations Office, under contract No. AC04-76ET33626.

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[Received July 15, 1986]