peptidase obtained from the Sephadex G-15 elution are shown in Table III. Ney (1971) reported that peptides with a Q value <1300 were not bitter and peptides with a Q value >1400 gave a bitter taste. The bitterness and Q value of fractions II and III apparently decreased in comparison with those of the bitter peptide fraction (fraction I). This result coincides with the prediction of bitterness of peptide described by Ney (1971). The more bitter peptides with a Q value >1400 in the bitter peptide fraction may be easily hydrolyzed by the enzyme and passed into dipeptide fraction (fraction III) and free amino acid fractions. The dipeptide fraction (fraction III), in which glutamic acid was enriched, is expected to have a masking effect for bitter taste as reported by Noguchi et al. (1972). As result of the enzymatic reaction, the bitterness of the bitter peptide fraction, on the whole, lessened.

It is concluded that wheat carboxypeptidase acts on the bitter peptides and releases hydrophobic amino acids from their carboxyl termini followed by a decrease in bitterness. Wheat carboxypeptidase may be useful for debittering protein hydrolysates in food application.

Registry No. Carboxypeptidase, 9031-98-5.

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Received for review October 22, 1981. Revised manuscript received August 6, 1982. Accepted September 10, 1982.

Redistribution of Oxamyl from Treated Seeds to Peach Seedlings and Soil As Determined by High-Performance Liquid Chromatography

Mikio Chiba,* Darrell F. Veres, John L. Townshend, and John W. Potter

Movement and degradation of oxamyl in peach seeds, seedlings, soil, and pots were studied. The determination of oxamyl and oxime was made by reverse-phase high-performance liquid chromatography using a UV detector; for analysis of seeds, soil, and pots no cleanup was required, but for roots and leaves cleanup procedures were essential. Peach seeds with endocarps removed were coated with oxamyl and planted individually in sterilized soil in clay pots. After 1 week, 5.7 and 1.4 ppm of oxamyl were found in the soil and clay pots, respectively. The corresponding oxime, methyl N-hydroxy-N',N'-dimethyl-1-thiooxamimidate, was also found, but intact oxamyl constituted 97% and 52% of the total residue in the soil and pots, respectively. There was no evidence of oxamyl degradation on the treated seeds 3 weeks after planting. Residues of oxamyl in leaves 2 and 3 weeks after sowing were 4.8 and 2.7 ppm, respectively; similarly, oxime residues were 4.3 and 4.8 ppm.

Oxamyl, methyl N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate, is known to control a wide range of insect and nematode pests (Bromilow, 1976). Although it is ambimobile (Peterson et al., 1978), it would be more practical to rely upon its downward movement for protection of a crop from nematodes because spray application to the foliage is easier than soil application of the pesticide.

However, application of a pesticide to seed would be more efficient and more practical than soil treatment. Seeds of cereals, vegetables, soybeans, cotton, and alfalfa have been treated with oxamyl (Prasad and Rao, 1976; Rodriquez-Kabana et al., 1977; Truelove et al., 1977; Hoveland et al., 1977; Townshend and Potter, 1979) to provide protection against nematodes during and after germination. So that the fate of oxamyl applied to individual seeds can be studied, a larger seed than those mentioned above is desirable; consequently, peach seeds with their endocarps removed were chosen for study.

If it is assumed that peach seeds were planted with endocarps removed, seed treatment with oxamyl should protect the seed and roots from nematode attack at the very beginning of root formation when roots are most susceptible to nematodes (Koch, 1955). Furthermore, the active ingredient taken up by the plant should work as an insecticide to protect the leaves.

In the past, several analytical methods for oxamyl residues, a GC method (Holt & Pease, 1976), a spectropho-

Agriculture Canada, Research Station, Vineland Station, Ontario, Canada L0R 2E0.

0021-8561/83/1431-0053$01.50/0 Published 1983 by the American Chemical Society

tometric method (Singhal et al., 1977), and a titrimetric method (Singhal et al., 1978) were reported, but none of those methods was suitable for individual determination of oxamyl and its corresponding oxime, methyl *N*-hydroxy-*N'*,*N'*-dimethyl-1-thiooxamimidate, which is produced as a result of degradation of oxamyl. More recently, two high-performance liquid chromatographic (HPLC) methods were reported which are applicable to detect 1-ppm level of residues. However, neither was practical because one was for the determination of ^{14}C-labeled oxamyl by a scintillation flow monitor after extensive cleanup procedures (Harvey et al., 1978) and the other was only for oxamyl, yet it yielded poor recoveries (Thean et al., 1978). Reported here is an improved HPLC method by which satisfactory recoveries can be obtained at or lower than the 0.1-ppm level. With this method, the movement of oxamyl from the seed into seedling tops and roots and into the soil and pot was investigated.

MATERIALS AND METHODS

Greenhouse Experiments. So that the fate of oxamyl applied to peach seeds (cv. Bailey) could be traced, the stony endocarps were removed and the seeds cold stratified at 5.5 °C for 11 weeks. Seeds were then soaked in an oxamyl solution (16 000 ppm) for 3 h at 23 °C, air-dried, and planted. Each treated seed was sown in sterilized soil (Vineland silt loam mixed with peat moss and washed sand at 10:6:3 v/v) in a small clay pot (5-cm diameter). Four treated seeds were analyzed before sowing to measure the initial deposit of oxamyl. One, two, and three weeks after sowing, each pot was emptied, and the seedling was divided into seed, root, and leaf (except 1 week after sowing when no leaf growth was observed) and individually analyzed; soil and pot were also analyzed. Four replicate treated and control samples were prepared for each sampling time.

Analytical Procedures. *Extractions.* Fifty grams of soil was placed in a 250-mL centrifuge container and 100 mL of methanol was added. The mixture was tumbled for 10 min and the container was centrifuged for 5 min at 2000 rpm; the supernatant was transferred to a 500-mL volumetric flask. The above procedures were repeated 4 more times, and the combined extracts were filtered through a 10-μm Millipore filter. The filtrate was ready for HPLC analysis.

A seed sample was soaked in 10 mL of methanol in a scintillation vial for 24 h, and the solution was treated with the sonifier for 4 min; during the 4-min period the vial was kept in a 250-mL beaker containing running cold water at 8–10 °C. The sonifier treatment was repeated with fresh 10 and 5 mL each of methanol to complete the extraction, and the extracts were passed through the Millipore filter as above.

A pot (weight about 70 g) was crushed into small pieces, and the pieces were transferred to a centrifuge bottle. After 100 mL of methanol was added to the bottle, the contents were kept at room temperature for 24 h. The contents were then tumbled for 10 min and centrifuged for 5 min. The tumbling and centrifuging procedures were repeated with 100 and then 50 mL of fresh methanol. The extracts were combined and filtered through a 10-μm Millipore filter and concentrated to the appropriate concentrations for HPLC analysis.

Root samples were soaked in 6 mL of methanol and kept at room temperature for 24 h, then treated with the sonifier for 4 min at 8–10 °C as stated above, and filtered through a 10-μm Millipore filter.

One gram of leaf sample was extracted with 25 mL of methanol for 30 min on the wrist-action shaker; this extraction procedure was repeated 3 more times with 25 mL each of methanol, and the methanol extracts were combined.

Cleanup. Cleanup of root and leaf extracts was required but not for the soil, seed, and pot extracts. To the root extract a drop of H_3PO_4 was added and the methanol was evaporated. The residue was dissolved in 1 mL of water, the solution was passed through a C-18 Sep-PAK column after a proper conditioning with 5 mL of methanol and 5 mL of distilled water, and the column was eluted with 6 mL of 20% methanol in water. The methanol was evaporated, and the residual aqueous phase was concentrated to 1 mL by a gentle stream of nitrogen; this solution was ready for injection into the Spectra-Physics HPLC system. From 100 mL of leaf extract, 50 mL was taken and 5 mL of 2% H_3PO_4 was added. The methanol was evaporated and the residual aqueous phase was transferred to a 60-mL separatory funnel to which 5 mL of *n*-hexane was added. After the mixture was shaken, the hexane layer was washed 4 times with 5-mL portions of 2% H_3PO_4 and all the aqueous phase were combined. The remaining hexane in the aqueous phase was removed by heating in a water bath, and the aqueous phase was passed through the XAD-2 resin (13 g in a column). The adsorbed oxamyl was eluted with 30 mL of methanol and collected in a 100-mL round-bottom flask; to the eluate 1 drop of H_3PO_4 was added and methanol was evaporated by using the rotary evaporator. The aqueous phase remaining was transferred to a small vial, to which the methanol rinsing of the flask was added, the methanol was evaporated by a gentle stream of nitrogen, and the residual aqueous phase was filtered through the Bioanalytical filtering system. The final volume of the aqueous phase was adjusted to 1 mL, and 0.5 mL of which was injected into the Milton-Roy HPLC system. The mobile phase used was 5% methanol in water and the flow rate was 1–1.5 mL/min. The oxamyl fraction was collected, a drop of H_3PO_4 added, and methanol in the eluate was evaporated in a water bath under a gentle stream of air. The aqueous phase that remained was then passed through the XAD-2 resin column. The material adsorbed in the column was eluted with 30 mL of methanol; after 1 drop of H_3PO_4 was added, the methanol was evaporated and the remaining aqueous phase was filtered through the Bioanalytical filtering system. The filtrate was adjusted to an appropriate volume and ready for injection onto the final HPLC analysis.

Recovery and Extraction Efficiency Tests. To a 50-g soil sample, 1 mL of 500 μg/mL oxamyl or 200 μg/mL oxime aqueous solution was added, and extraction and further analytical procedures were pursued as described above and in the following HPLC procedures. To a 1-g root sample, 0.1 mL of 100 μg/mL oxamyl or oxime aqueous solution was added at the 10-ppm level, and the above extraction and cleanup procedures were pursued as above. To a leaf sample, oxamyl or oxime was added at the 5- and 1-ppm level, respectively, and the above extraction and cleanup procedures were followed as described above. Regarding seed and pot samples, the exhaustive extraction approach using actually treated samples was taken rather than ordinary recovery procedures because much more realistic data were expected by this approach. Extractions were repeated as described in the above extraction procedure until no more residues of oxamyl or oxime were found in each of the extracts, and the sum of the quantities extracted were expressed as 100% of the residues present at the time of analysis.

High-Performance Liquid Chromatograph (HPLC). Spectra-Physics Model SP-8000 HPLC equipped with Spectra-Physics autoinjector and Spectra-Physics SP8300

UV–vis fixed-wavelength detector was used for final analysis. Also, a Milton-Roy HPLC system, equipped with 5000-psi minipump, was used mainly for cleanup of sample extracts. Spectra-Physics SP8200 UV–vis fixed-wavelength detector was used for this HPLC system. The wavelength used was 254 nm with both detectors. Sample loops used were 25 μL for the SP-8000 system and 500 μL for the Milton-Roy system.

The analytical columns used were a Brownlee Lichrosorb C-18 column, 4.6 mm (i.d.) × 25 cm for soil, or a Spectra-Physics C-8 column, 4.6 mm (i.d.) × 25 cm for seed, pot, and root, in conjunction with an MPLC guard column, 4.6 mm (i.d.) × 3 cm, packed with the same Lichrosorb C-18 10-μm packing. Homemade columns, packed with Shandon ODS-Hypersil C-18 packing, 5 μm, 4.6 mm (i.d.) × 12 cm, in conjunction with the MPLC guard column, were used for leaf samples. For cleanup, a Waters Radial-PAK cartridge column, C-18, 10 μm, 8 mm (i.d.) × 10 cm, in conjunction with the radial compression separation system, was used. The mobile phases used were 1–20% methanol in water and run isocratically for soil, seed, pot, and root analyses. For leaf samples, gradient systems were empolyed with ternary systems composed of methanol, acetonitrile, and water.

Chemicals. *Oxamyl and Corresponding Oximino Compound.* Analytical standards of those were obtained from E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898. Vydate L (24% oxamyl a.i. water-soluble liquid) was used for treatment. Oxamyl is classified Class B poison under I.C.C. regulation.

Solvents. Acetonitrile, methanol, and hexane were all HPLC grade from Caledon Laboratories, Ltd., Georgetown, Ontario, Canada L7G 4R9.

Polymeric Adsorbent Beads. Polystyrene XAD-2 Resins (catalogue No. B55088-34) were obtained from BDH Chemicals, Toronto, Ontario, Canada M8Z 1K5.

Sep-PAK Cartridge. C-18 cartridges (part no. 51910) were from Waters Scientific Ltd., Mississauga, Ontario, Canada L4V 1H3.

Millipore Filter. A 10-μm LC-type filter (LCWP 02500), Millipore Corp., Bedford, MA 01730, was used.

Microfilter. A 0.2-μm nitrocellulose filter (Bioanalytical Systems, Inc., West Lafayette, IN 47906) was used.

ODS-Hypersil, 5-μm, was from Shandon Southern Instruments, Inc., Sewickley, PA 15143.

Apparatus. *Centrifuge.* An MSE GF-6 centrifuge, Johns Scientific, Toronto, Ontario, Canada M4M 2G4, was used.

Tumbler. The Fisher-Kendall mixer was from Fisher Scientific Co., Don Mills, Ontario, Canada M3A 1A8.

Shaker. A Burrell wrist action shaker, Model 75 (Burrell Corp., Pittsburgh, PA), was used.

Rotary Evaporatory. A Buchê rotavapor (R), from Canadian Laboratory Supplies (Canlab), Toronto, Ontario, Canada M8Z 2H1, was used.

Sonifier (Ultrasonic Disintegrator and Homogenizer). The sonifier was a Biosonik sonifier, from Bronwill Scientific (Division of Will Scientific, Inc.), Rochester, NY.

Radial Compression Separation System (RCSS). This was obtained from Waters Scientific, Ltd., Mississauga, Ontario, Canada L4V 1H3.

RESULTS AND DISCUSSION

Recovery. With the method described, satisfactory recoveries or extraction efficiencies of oxamyl and oxime were obtained (Table I). Because the ordinary fortification procedure was impractical with seeds and pots, extractions were repeated exhaustively with the actual samples. Oxime was also found in these seeds because the commercial product of Vydate, used in this experiment, contained a small quantity of oxime: 1.8% of the quantity of oxamyl. Recoveries from root, soil, and leaf were very good with the lowest value of 90.4% for oxime in leaf. With the exhaustive extraction method, the values were expressed as 100% because the extraction was repeated until no more residues of oxamyl or oxime were found.

Table I. Efficiency of Recoveries (Percent) of Oxamyl and Oxime When Added to Soil, Root, Leaf, Seed, and Pot Samples[a]

	oxamyl		oxime	
sample	levels added, ppm	recovery, %	levels added, ppm	recovery, %
soil[b]	10	98.6 ± 0.6[d]	4	92.1 ± 2.8
root[b]	10	98.5 ± 3.7	10	98.1 ± 2.8
leaf[b]	5	95.2 ± 3.3	1	90.4 ± 0.2
seed[c]	NA[c]	100.0[e]	NA	100.0[f]
pot[c]	NA	100.0	NA	100.0

[a] Each figure represents the average of three determinations. [b] Known quantities of oxamyl or oxime were added to the sample directly. [c] No oxamyl or oxime was added because actually treated samples were used. [d] Standard deviation. [e] Total quantities found were expressed as 100%. [f] Recovered only from treated seeds before planting.

Cleanup and HPLC Analysis. The reproducibility of HPLC analyses was good, and the lowest detectable limit was 0.1 ppm for oxamyl and 0.05 ppm for oxime in all the samples. The detector response was linear at least in the range of 0.05 and 40 ppm. Originally, development of a simple method to determine oxamyl and oxime simultaneously without cleanup was intended as previously demonstrated with benomyl and MBC (Chiba and Veres, 1980, 1981). Leaf extracts, however, showed very strong interference on the UV detector and their analysis was impossible without cleanup. We tried a Sep-PAK column, but it was not strong enough to remove all the impurities. A satisfactory cleanup with good recoveries was obtained only after the XAD-2 column cleanup and RCSS HPLC cleanup.

Although Davies et al. (1978) did not need any cleanup for their leaf analysis, their concentrations were much higher and they simply washed off the surface deposits. Harvey et al. (1978) used extensive cleanup procedures with [^{14}C]oxamyl samples. Thean et al. (1978) also used extensive cleanup procedures for vegetables; they claim their cleanup was satisfactory, but the recoveries were rather low, being 61–77%. In addition, they did not intend to analyze oxime. The cleanup procedure reported here is applicable for determination of both oxamyl and oxime, and recovery values were substantially better than those reported by Thean et al. (1978) as shown in Table I.

During the course of study, the percentage of methanol present in water when injection was made was found to be very critical for the obtainment of reproducible results in the peak height of oxamyl and oxime in HPLC analyses. Although the retention time was unchanged, the peak height became substantially smaller when the percentage of methanol in water increased (Figure 1). The sensitivity of both oxamyl and oxime was almost doubled by increasing the oven temperature from 25 to 65 °C, but it was found that the use of a high temperature was not a real advantage with the sample analyses: as the temperature increased, the resolution between oxamyl or oxime and impurity peaks rapidly deteriorated.

Actual Residues. The amount of oxamyl, initially adsorbed by the seeds after 3 h of soaking, ranged from 386 to 741 μg (Table II). Although treated seeds before

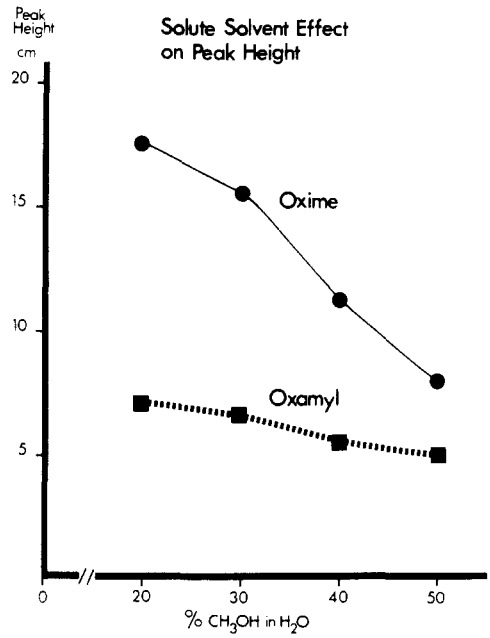

Figure 1. Influence of compositions of the mobile phase (volume/volume percent of methanol in the methanol and water system) on peak heights of oxamyl and oxime (Brownlee C-18 column; oxamyl and oxime were both at 4 ppm).

Table II. Initial Intake of Oxamyl in Seeds after 3 h of Soaking in an Oxamyl Solution (16 000 ppm) at 23 °C

seed no.	seed wt, mg	initial total intake of oxamyl/seed, μg	μg of oxamyl/g of seed, ppm
1	663.0	386.0	582.2
2	804.0	614.0	763.7
3	650.0	555.4	854.5
4	847.0	740.8	874.6
av	741.0	574.1	768.8
SD	99.3	147.3	133.4

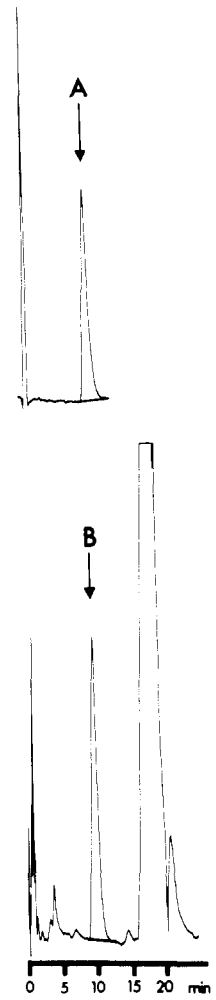

Figure 2. Chromatograms of standard oxamyl and seed extract: (A) standard 10 ppm of oxamyl; (B) treated seed extract without cleanup.

sowing contained oxime as discussed previously and shown in Table I, planted seeds did not show any trace of oxime 1, 2, and 3 weeks after sowing. In contrast, fairly substantial quantities of oxamyl were found in all the seed samples even after 3 weeks (Table III). Residues of both oxamyl and oxime were found in most other samples. As oxamyl concentrations gradually declined, oxime concen-

Table III. Average Concentrations[a] (ppm) of Oxamyl and Oxime in Soil, Seed, Pot, and Leaf Samples, 1, 2, and 3 Weeks after Sowing

sampling time after sowing, week(s)	soil		seed		pot		leaf	
	oxamyl	oxime	oxamyl	oxime	oxamyl	oxime	oxamyl	oxime
1	5.70 ± 0.47[b]	0.17 ± 0.13	6.67 ± 3.01	ND[c]	1.43 ± 0.35	1.30 ± 0.26	NA[d]	NA
2	2.25 ± 0.53	0.24 ± 0.05	2.55 ± 0.90	ND	0.74 ± 0.29	1.48 ± 0.36	4.75 ± 2.83	4.25 ± 2.46
3	0.78 ± 0.55	0.42 ± 0.23	1.60 ± 1.40	ND	0.16 ± 0.32	2.75 ± 0.74	2.67 ± 1.27	4.80 ± 1.71

[a] Average of four replicate samples. [b] Standard deviation. [c] Not detected at the 0.05-ppm level. [d] No leaf grown yet.

Table IV. Total Quantities of Oxamyl and Oxime Recovered from All the Experimental Media Including Soil, Seed, Pot, and Leaf[a]

sampling time after sowing, week(s)	initial wt of seeds, mg	initial quantities of oxamyl adsorbed in seeds,[b] μg	recovered quantity			
			as oxamyl and oxime		as oxamyl	
			μg	% recovery	μg	% recovery
1	783 ± 67[c]	626 ± 83	511 ± 86	81.6	402 ± 48	64.2
2	836 ± 94	695 ± 116	337 ± 65	48.5	180 ± 46	25.9
3	884 ± 267	751 ± 330	347 ± 88	46.2	55 ± 50	7.3

[a] Each value represents the average of four replicate sample analyses. [b] Calculated values, obtained from the following equation: $y = 1.236x - 342.05$, where y is the quantity of oxamyl expected to be adsorbed by a seed and x is the weight of seed (the equation was obtained from the data shown in Table III and the correlation coefficient is 0.833). [c] Standard deviation.

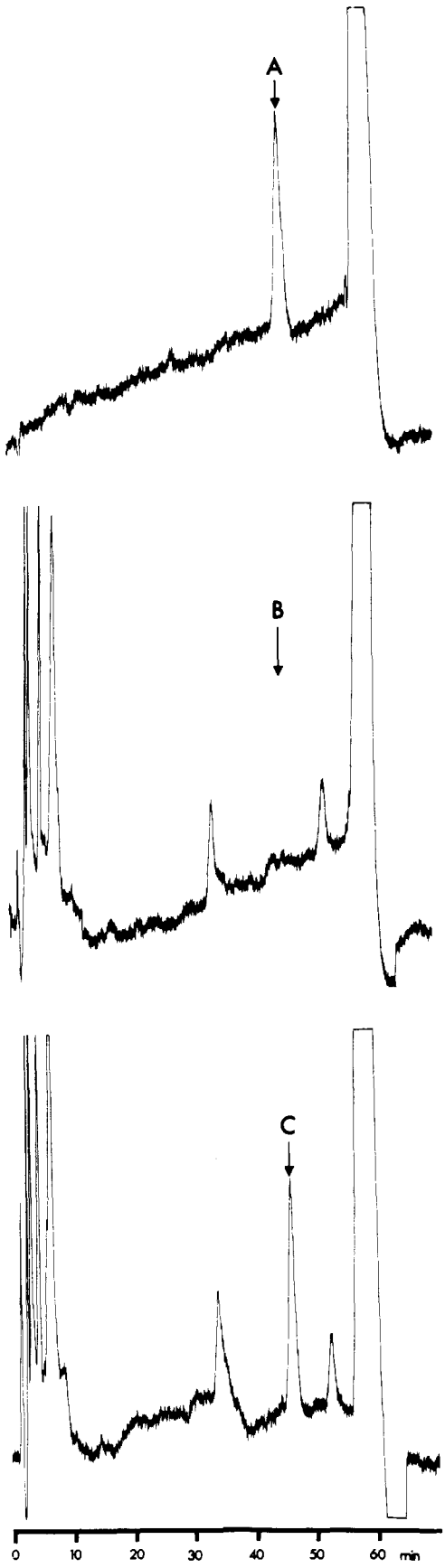

Figure 3. Chromatograms of standard oxime and leaf extracts: (A) standard 1 ppm of oxime; (B) untreated leaf extract after cleanup; (C) combination of (A) plus (B) (1 ppm of oxime prepared in untreated leaf extract after cleanup).

trations consistently increased in soil, pot, and leaf samples (Table III). Neither compound was detected in root samples over the experimental period of 3 weeks. This could be due to small quantities of root samples available from single plants for individual analyses (0.5–2 g). Figure 2 shows chromatograms of standard oxamyl and seed extract without cleanup, and Figure 3 shows chromatograms of standard oxime, untreated leaf extract after cleanup, and the combination of both.

The presence of other degradation products as observed in plants previously (Harvey et al., 1978) was also suggested by the results of this study because less than 50% of oxamyl initially available in seeds was recovered as oxamyl and oxime from the experimental media after 2 and 3 weeks (Table IV). However, no effort was made to identify those degradation compounds, because their biological activity has not been identified to date.

The lateral movement of oxamyl in soil was clearly demonstrated in this experiment. In the past, the influence of soil moisture on the half-life of oxamyl (Bromilow and Baker, 1980), the effect of organic matter on the fate of oxamyl (Harvey and Han, 1978), and the downward movement (Leistra et al., 1980) in soil were investigated; the finding of the lateral movement, particularly in the form of intact oxamyl, was significant. This movement may have been enhanced by evaporation of water from the clay pot, which induced the movement of oxamyl along with the water added to the soil. It appears that oxamyl degraded to oxime much faster in clay pots than in soil.

Relatively high concentrations of oxamyl were found in leaf samples (Table III). This indicates that seed treatment with oxamyl may be a good means of protecting the foliage of plants against insects since it is a good insecticide.

In a earlier study by Bunt and Noordink (1977), the downward movement of ^{14}C radioactivity in a plant was confirmed after a foliar application of ^{14}C-labeled oxamyl, but the finding of intact oxamyl in roots was inconclusive. The presence of biological activity in or around root, in protecting roots from nematode attack when foliar applications of oxamyl were made, was well demonstrated (Potter and Marks, 1976), but no other biologically active component has been identified from roots to date. Further studies on the fate of oxamyl and the practicality of coating peach seeds with oxamyl for protection of roots from nematodes are in progress.

Registry No. Oxamyl, 23135-22-0; methyl *N*-hydroxy-*N'*,*N'*-dimethyl-1-thiooxamimidate, 30558-43-1.

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Received for review March 3, 1982. Revised manuscript received August 9, 1982. Accepted September 9, 1982. This paper was presented at the 182nd National Meeting of the American Chemical Society, Division of Pesticide Chemistry, New York, NY, Aug 1981.

A Simple Turbidimetric Method for Determining the Fat Binding Capacity of Proteins

Leandros P. Voutsinas and Shuryo Nakai*

A simple turbidimetric method was developed for determining the fat binding capacity (FBC) of various proteins. The turbidity was dependent on wavelength, blending time, and volume of oil. The FBC was positively affected by surface hydrophobicity (S_0) and negatively affected by the interaction of S_0 with solubility (s). A highly significant correlation ($R^2 = 0.802$, $P < 0.01$) was found between S_0, $S_0 \times s$, and FBC of 11 food proteins tested. Advantages of the method developed include a small amount of sample required for FBC determination and the fact that the measured values would reflect the true fat binding capacity of proteins by minimizing the fat-entrapping effects.

The ability of proteins to bind fat is an important functional property for such applications as meat replacers and extenders, principally because it enhances flavor retention and reputedly improves mouth feel (Kinsella, 1976). The key role of fat in food flavoring has been demonstrated by Kinsella (1975), and its capacity to improve flavor carry-over in simulated foods during processing is apparent. Soy proteins have been added to ground meats to promote fat absorption or fat binding and thus decrease cooking losses and maintain dimensional stability in the cooked product (Wolf and Cowan, 1975). Fat separation is a well-known major problem in processed meat-in-sauce-or-gravy type products. This problem can be prevented by incorporating into these products (canned or frozen meat/sauce products) a combination of soy protein ingredients (i.e., an extruded soy protein concentrate, a soy protein isolate, and lecithin) designed to emulsify, bind, and stabilize fats (Morris, 1980). On the other hand, in some foods such as pancakes and doughnuts, the addition of soy flour helps to prevent excessive absorption during frying (Johnson, 1970).

Fat absorption of proteins is usually measured by adding excess liquid fat (oil) to a protein powder, thoroughly mixing and holding, centrifuging, and determining the amount of bound or absorbed oil, which is total minus free (Lin et al., 1974; Wang and Kinsella, 1976). The amount of oil and protein sample, kind of oil, holding and centrifuging conditions, and units of expression have varied slightly from one investigator to another (Hutton and Campbell, 1981).

The mechanism of fat absorption is not clear. However, Wang and Kinsella (1976) have attributed fat absorption, as assessed by the above method, mostly to physical entrapment of the oil; in support of this a correlation coefficient of 0.95 was found between bulk density and fat absorption by alfalfa leaf proteins. Chemical modification of protein, which increases bulk density, concomitantly enhances fat absorption (Franzen, 1975).

The objective of this study was to develop a simple method for determining the ability of proteins to bind fat. An effort was made to avoid the entrapment of oil by proteins in order to measure the amount of oil truly bound to the proteins. The development of the method and a comparison of the fat binding capacities of several food proteins are presented.

MATERIALS AND METHODS

Materials. Bovine serum albumin (No. A-4503), β-lactoglobulin (No. L-6879 from milk), and ovalbumin (No. A-5503) were all purchased from Sigma Chemical Co., St. Louis, MO. Soy protein isolate was obtained from General Mills, Inc., Minneapolis, MN. Promine D was purchased from Central Soya Co., Chicago, IL. Pea protein isolate (M 412-046), Century cultivar field pea, was received from POS Pilot Plant Corp., University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Rapeseed protein isolate and sunflower protein isolate were prepared by the method of Nakai et al. (1980). Gelatin, Bloom 300, was purchased from United States Biochemical Corp., Cleveland, OH. Whey protein concentrate (75%) was obtained from Sodispro Technology, St. Hyacinthe, Quebec, Canada. Whole casein was prepared by the method of Voutsinas et al. (1982). Corn oil was from Fisher Scientific Co., Fair Lawn, NJ. Urea, ACS reagent, 99+%, was obtained from Aldrich Chemical Co. Inc., Milwaukee, WI. Metaphosphoric acid was from J. T. Baker Chemical Co., Phillipsburg, NJ.

Fat Binding Capacity Determination. To 40 mg of freeze-dried protein sample in a 15-mL glass centrifuge tube 1.5 mL of corn oil was added. The contents were stirred and sonicated, with a Braun-Sonic 1510 sonicator (Braun Instruments, San Francisco, CA) fitted with a needle probe, at 100 W for 1 min to disperse the protein sample. After being held at room temperature for 30 min the tube was centrifuged at 3020g for 20 min. The free oil was pipetted off and 2 mL of distilled water was added. Oil adhered to the sides of the tube was removed by

Department of Food Science, The University of British Columbia, Vancouver, British Columbia, Canada V6T 2A2.

0021-8561/83/1431-0058$01.50/0