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Determination of Tryptophan in Unhydrolyzed Food and Feedstuffs by the Acid Ninhydrin Method

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The optimum conditions for direct measurement of tryptophan in soybean meals, wheat, maize, barley, rye, sunflower, fish, and meat meals have been reported. The acid ninhydrin solution has been reacted directly with the protein matrix without any previous isolation or dissolution of the protein. Reproducibility and recovery studies showed that 10–100 μg of tryptophan-containing protein can be determined with a standard error of 2.7% or less. The decreases of tryptophan content of treated soybeans are essentially a function of the parameters applied. The remaining tryptophan contents, expressed in the percentages of the untreated one, were as follows: 81% HCl + H_3PO_4 (pH ~1.8), neutralized; 79% HCl + H_3PO_4 (pH ~2.0), neutralized; 67% HCl + H_3PO_4 (pH ~2.0), without neutralization; 63% microwave 5 min, 100 °C; 57% HCl (pH ~2.0), without neutralization.

Finding a really reproducible tryptophan assay is still a relevant task in protein research (Cuq et al., 1983; Cuq and Cheftel, 1983; Finot et al., 1982; Friedman and Finley, 1971; Friedman et al., 1984; Friedman, 1984; Friedman and Cuq, 1988; Nielsen et al., 1985a,b). It is well-known that, under the common parameters of protein hydrolysis (6 mol of HCl, 100 °C, 24 h), a considerable amount of tryptophan became destroyed because of the particular reactivity of the indole ring (Fontana, 1984).

To avoid tryptophan losses, time-consuming and tedious special hydrolysis conditions have been suggested in the literature: Several procedures are based on hydrolyses by hydrochloric acid with additives (Andersen et al., 1984; Ashworth, 1987; Felker, 1976; Gardner, 1984; Gruen and Nicholls, 1972; Matsubara and Sasaki, 1969; Ohta and Nakai, 1979; Wong et al., 1984), by organic acids (Csapó and Csapó-Kiss, 1985; Csapó and Csapó-Kiss 1986; Csapó et al., 1986; Gundel and Votisky, 1974; Liu and Chang, 1971; Penke et al., 1974; Simpson et al., 1976), or by bases (Allred and MacDonald, 1988; Buttery and Soar, 1975; Delhaye and Landry, 1986; Gorinstein et al., 1988; Huet and Pernollet, 1986; Hugli and Moore, 1972; Kirchgessner et al., 1987; Knox et al., 1970; Landry et al., 1988; Levine, 1982; Lucas and Sotelo, 1980; Nielsen et al., 1985a,b; Nielsen and Hurrel, 1984, 1985; Piombo and Lozano, 1980; Sato et al., 1984, 1987; Werner, 1986; Williams et al., 1982).

Less emphasis have been focused on the so-called direct methods that do not need the previous hydrolysis of proteins (Barman and Koshland, 1967; Chrastil, 1986; Concon, 1975; Dickman and Crockett, 1956; Friedman and Sigel, 1966; Friedman, 1967; Gaitonde and Dovey, 1970; Iizuka and Yajima, 1985; Nakae and Shono, 1984; Opienska-Blauth et al., 1963; Servillo et al., 1982; Sodek et al., 1975; Votisky, 1984; Zahnley and Davis, 1973).

As a result of earlier comparative studies, it has been stated (Friedman et al., 1984) that "the acid ninhydrin method, merits further study to assess its general applicability".

Thus, recently, exhaustive investigations have been carried out to find optimum conditions for the direct tryptophan assay to improve the acid ninhydrin method (Molnár-Perl and Pintér-Szakács, 1989).

As a result of kinetic studies (Molnár-Perl and Pintér-Szakács, 1989) it has been proven that excellent results can be achieved at 35 °C after 2-h reaction time, with 1% ninhydrin containing acidic reagent (96% formic acid/cc. HCl = 3/2; detailed composition in Materials and Methods (i) without the disturbing effect of tyrosine reported by Zahnley and Davies (1973) and (ii) without the necessity of previous isolation (Sodek et al., 1975) or dissolution (Gaitonde and Dovey, 1970) of the protein content of the sample to be analyzed.

In this paper we show the applicability of the acid ninhydrin method in determination of the tryptophan content both in intact food and feedstuffs and in differently treated soybean meals.

MATERIALS AND METHODS

Materials. Bovine serum albumin 1, human serum albumin 1, and α-chymotrypsin were obtained from Reanal (Buda-

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Table I. Absorbance Values of Various Amounts of Tryptophan and Lysozyme (Expressed as Tryptophan) Obtained from Solutions Diluted in Different Ways

compn of measd solns	abs values,[a] reacted Trp, μg/10 cm³				mean[b]	std error	std error, %	$A_m \times 10^{-3}$ [c]/M·cm
	10	20	40	100				
R_n:D = 1:4	0.112	0.222	0.444	1.133	0.555	0.0076	1.4	22.2
R_n:D = 1:1	0.112	0.229	0.456	1.135	0.568	0.0054	1.0	23.2
R_n:E = 1:4	0.112	0.224	0.452	1.133	0.563	0.0034	0.6	23.0
R_n:E = 1:1	0.115	0.224	0.447	1.135	0.565	0.0075	1.3	23.0
R_n:DMF = 1:4	0.113	0.225	0.460	1.132	0.567	0.0055	1.0	23.2
R_n:DMF = 1:1	0.113	0.230	0.452	1.135	0.568	0.0047	0.8	23.2

compn of measd solns	reacted lysozyme, μg				mean[b]	std error	std error, %	$A_m \times 10^{-3}$ [c]/M·cm
	10	25	50	100				
R_n:D = 1:4	0.085	0.220	0.440	0.875	0.436	0.0087	2.0	17.8
R_n:D = 1:1	0.088	0.218	0.440	0.880	0.439	0.0023	0.5	17.9
R_n:E = 1:4	0.085	0.222	0.438	0.878	0.437	0.0081	1.9	17.8
R_n:E = 1:1	0.090	0.222	0.435	0.882	0.443	0.0063	1.4	18.1
R_n:DMF = 1:4	0.088	0.220	0.444	0.879	0.438	0.0083	1.9	17.9
R_n:DMF = 1:1	0.086	0.219	0.440	0.880	0.437	0.0048	1.1	17.8

[a] The mean of at least three measurements. [b] Mean, calculated from absorbance values of 10, 20, 40, and 100 μg of tryptophan/10 cm³ or 10, 25, 50, and 100 μg of tryptophan (in lysozyme)/10 cm³ and expressed for the solutions of 50 μg/10 cm³, in both cases. [c] A_m = molar absorptivity value = $A_m \times 10^{-3}$/M·cm; D = distilled water; E = ethyl alcohol; DMF = dimethylformamide.

Table II. Time Requirement for the Quantitative Interaction of Various Reactants under Optimum Analytical Conditions

sample	Trp content, % of value obtained after 2-h reaction at reaction time (min)						
	10	30	60	90	120	150	180
soybean meal (extracted)	20	90	96	100	100	100	105
soybean meal (crude)	56	64	79	98	100	101	106
wheat	76	76	79	99	100	102	104
maize	65	93	100	100	100	100	105
barley	*a*	52	92	100	100	100	100
rye	*a*	66	81	97	100	102	104
sunflower	56	91	93	95	100	102	104
fish meal	32	45	65	87	100	100	100
meat meal	62	68	71	87	100	105	120

[a] Not filterable.

pest, Hungary). Bovine serum albumin 2, human serum albumin 2, and trypsin came from Sigma (St. Louis, MO). Human γ-globulin and casein were from Serva (Heidelberg, F.R.G.) (their collective designation is standard proteins). Food and feedstuffs, commercial products of different origins, as well as variously treated soybeans and diets for broilers were obtained partly from the State Office of Technical Development of Hungary and partly from the Hajduság Agrarian Industrial Co.

All chemicals used were of analytical reagent grade. Reagents were prepared with analytical precision by mixing concentrated hydrochloric acid (Merck, p.a. 37%) with ~96% (m/v) formic acid in a volume ratio of hydrochloric acid to formic acid of 2/3. (The ~96% (m/v) formic acid was obtained from formic acid (Reanal p.a. 99%) and distilled water by mixing them in the volume ratio of 95/4.) The ninhydrin-containing reagent (R_n) was prepared by dissolving 1.0 g of ninhydrin (weighed with analytical precision) in R_0 in a 100-mL volumetric flask (R_0 = ninhydrin-free reagent), and after dissolution the same R_0 was used to adjust the volume to 100.00 mL.

Procedure. All reactions were carried out at 35 °C (±0.05 °C), and solutions were previously thermostated on an electrical shaker with 24 places (Vibroterm, Labor MIM, Budapest, Hungary). From 10 to 100 μg of tryptophan or lysozyme (equivalent to 10–100 μg of tryptophan) was added to 2 or 5 mL of R_n previously thermostated in a 10-mL volumetric flask. The sparingly soluble proteins were shaken under constant temperature. After the chosen reaction time, generally after 2 h, the solutions were cooled to room temperature and ethyl alcohol (E) or dimethylformamide (DMF) was added to bring the volume to 10.0 mL. The contents of the 10-mL volumetric flask, if necessary, were filtered through a filter cap with fiber glass filter paper (Grade GF/A, Whatman, Great Britain), and, if not otherwise stated, 1 mL of the supernatant was pipetted to a 5-mL volumetric flask and the corresponding solution added to bring its volume to 5.00 mL. (The corresponding solutions were as follows (expressed in volume ratios): R_0/E = 1/4 or 1/1; R_0/DMF = 1/4 or 1/1. (See also the data in Table I.)

Absorbance measurements were carried out with use of a Spectromom 195/D instrument (MOM, Budapest, Hungary). Matched quartz cells of 1-cm path length were used. The baseline and the digital absorbance readings were adjusted to 0.000 by keeping the reagent medium R_0 in both the reference and the sample chambers before each set of measurements. The absorbances were measured at 380 nm against the corresponding reagent blanks. The food and feedstuffs and the diet materials were reacted both with ninhydrin-containing reagent (absorbance values *A*) and with ninhydrin-free reagent (absorbance values *B*). The tryptophan contents have been calculated from the differences between the appropriate absorbance values (*A* − *B*).

RESULTS AND DISCUSSION

Based on our exhaustive kinetic study with tryptophan, lysozyme, and tyrosine (Molnár-Perl and Pintér-Szakács, 1989), it became clear that the acid ninhydrin/protein interactions can be achieved without the disturbing effect of tyrosine.

Continuing our research with food and feedstuffs revealed that those conditions proving to be perfect for the analysis of tryptophan and soluble proteins are not applicable without modification for the sparingly soluble protein matrices.

As a result of dilution of the supernatant with distilled water, following the filtration of the interacted reactants, insoluble precipitates have been formed.

To avoid the formation of insoluble particles, instead of using distilled water for dilution, a number of sol-

Table III. Reproducibility of the Determination of Tryptophan of Different Amounts of Standard Proteins in Solutions Diluted with Ethyl Alcohol (E) and Dimethylformamide (DMF)[a]

protein	amt reacted, mg	soln	A at 380 nm	g Trp/100 g protein		std error	std error, %
				single	mean		
human serum albumin 1	20.8	E	0.111	0.307			
	40.8		0.219	0.309			
	60.7		0.330	0.312			
	19.6	DMF	0.103	0.302	0.309 (1.0/1.0)	0.00443	1.4
	39.9		0.215	0.309			
	60.2		0.330	0.315			
human serum albumin 2	20.4	E	0.134	0.348			
	41.0		0.270	0.378			
	62.0		0.409	0.379			
	20.6	DMF	0.140	0.391	0.384 (1.2/1.0)	0.00639	1.7
	40.4		0.275	0.391			
	62.8		0.423	0.387			
bovine serum albumin 1	29.3	E	0.360	0.731			
	44.0		0.550	0.718			
	19.0	DMF	0.240	0.726	0.726 (2.3/2.0)	0.004911	0.7
	40.8		0.516	0.726			
	60.8		0.770	0.729			
bovine serum albumin 2	21.6	E	0.230	0.612			
	42.0		0.460	0.629			
	61.3		0.670	0.628			
	22.1	DMF	0.240	0.624	0.628 (2.0/2.2)	0.00993	1.6
	41.4		0.460	0.638			
	61.6		0.685	0.639			
human γ-globulin	1.036	E	0.243	2.71			
	1.830		0.430	2.70	2.69 (19.5/21)	0.0435	1.6
	2.120	DMF	0.490	2.66			
trypsin (bovine)	0.400	E	0.126	0.362			
	0.800		0.250	0.359			
	1.000		0.318	0.366			
	1.200		0.380	0.364			
	0.400	DMF	0.128	0.368	0.366 (4.2/4)	0.00381	1.0
	0.800		0.256	0.368			
	1.000		0.322	0.370			
	1.200		0.388	0.369			
α-chymotrypsin (bovine)	0.303	E	0.164	6.22			
	0.606		0.333	6.32			
	0.909		0.509	6.43			
	0.303	DMF	0.171	6.48	6.37 (7.2/7)	0.0919	1.4
	0.606		0.338	6.41			
	0.909		0.500	6.37			

[a] Applying the volume ratios of R_n or R_0/E or DMF = 1/1. Moles of tryptophan measured/moles of tryptophan theoretical, calculated with the molecular weights as follows, given in parentheses: human and bovine serum albumins, 66 000; human γ-globulin, 150 000; trypsin (bovine), 23 800, α-chymotrysin (bovine), 23 000.

vents have been tested (R_0, ethyl alcohol, formamide, dimethylformamide, acetonitrile, etc.).

In all cases investigated, R_0, ethyl alcohol, and dimethylformamide proved to be of the same value. Nevertheless, ethyl alcohol and dimethylformamide have been chosen as diluting agents to avoid working with the concentrated acidic solutions.

The reproducibility study of absorbance values, performed with 10–100 μg of tryptophan or lysozyme (expressed in tryptophan), under various dilutions has been summarized in Table I. Results showed that the molar absorptivity values ($A_m \times 10^{-3}$/M·cm) are not affected by the quality or by the volume ratios of the diluting solutions and the molar absorptivity values of tryptophan are higher (23.0×10^3/M·cm) than those of lysozyme (17.9×10^3/M·cm) expressed in tryptophan. Thus, for the tryptophan assay of food and feedstuffs in calibrating solution, lysozyme is to be used.

The optimum reaction time also for food and feedstuffs has been tested (Table II). In all cases investigated, 2 h proved to be the optimum. After a longer period of time (150–180 min) side reactions take place (100–105%), particularly in the case of meat meal after 3 h (120%).

The absorbances of tryptophan and standard proteins, as well as of food and feedstuffs, obtained with both solutions R_0 and R_n, reveal the following: The absorbance values of tryptophan at 390 nm and those of tryptophan residues of standard proteins at 380 nm, given by R_0, are negligible. I.e., in order to measure the tryptophan content of calibrating solutions or of standard proteins (Tables I and III) only reactions with R_n are to be performed and for calculations only readings A are to be considered. In the case of food and feedstuffs, reactions with both solutions R_n (readings A) and R_0 (readings B) are to be carried out, and the tryptophan contents of these samples are to be calculated from the differences of readings reached at 380 nm (Table IV, $A - B$ values).

From the data of Table IV it can be seen that the differences of absorbance values (readings $A - B$) are proportional to the amount of tryptophan present and the reproducibility of measurements of various amounts of protein matrices is satisfactory. The standard error percents of determinations vary from 0.7 to 2.7%.

The utility of the acid ninhydrin method is shown by the agreement of the tryptophan content of some food and feedstuffs measured by us and those found in the literature (Table V), by the recovery of tryptophan of single ingredients done in diets (Table VI), and by monitoring the transformation of tryptophan in soybeans under various denaturation processes (Table VII).

Table IV. Reproducibility of the Tryptophan Determinations of Various Amounts of Food and Feedstuffs in Solutions Diluted with Ethyl Alcohol (E) and Dimethylformamide (DMF)

sample	amt, mg	A at 380 nm soln	A	B	A − B	g Trp/100 g dry matter single	mean	std error	std error, %
soybean meal (extracted)	20.2	E	0.268	0.045	0.223	0.634			
	31.2		0.408	0.063	0.345	0.635			
	60.2		0.772	0.125	0.647	0.618	0.626	0.00874	1.4
	12.6	DMF	0.174	0.038	0.136	0.620			
	28.4		0.390	0.079	0.311	0.629			
	60.4		0.770	0.100	0.650	0.618			
soybean meal (crude)	20.2	E	0.234	0.050	0.184	0.524			
	31.1		0.374	0.090	0.284	0.525	0.522	0.00435	0.8
	60.4		0.723	0.180	0.543	0.517			
wheat	52.4	E	0.170	0.042	0.128	0.140			
	75.0		0.230	0.050	0.180	0.138			
	105.1		0.335	0.082	0.253	0.138	0.137	0.00272	2.0
	50.5	DMF	0.153	0.035	0.118	0.134			
	75.5		0.235	0.060	0.175	0.133			
	100.9		0.330	0.090	0.240	0.136			
maize	60.2	E	0.168	0.068	0.100	0.0954			
	100.8		0.265	0.100	0.165	0.0941			
	29.8	DMF	0.095	0.045	0.050	0.0964	0.0956	0.00103	1.1
	62.4		0.178	0.073	0.105	0.0967			
	90.5		0.280	0.130	0.150	0.0952			
barley	48.8	E	0.169	0.062	0.107	0.126			
	75.6		0.246	0.080	0.166	0.126			
	98.4		0.348	0.130	0.218	0.127	0.129	0.00346	2.7
	49.7	DMF	0.150	0.040	0.110	0.127			
	74.2		0.240	0.070	0.170	0.132			
	98.6		0.330	0.100	0.230	0.134			
rye	50.9	E	0.130	0.058	0.072	0.0813			
	74.2		0.181	0.080	0.101	0.0782			
	103.2		0.263	0.122	0.141	0.0785	0.0798	0.0146	1.8
	50.9	DMF	0.130	0.060	0.070	0.0790			
	73.9		0.190	0.085	0.105	0.0817			
	97.1		0.235	0.100	0.135	0.0799			
sunflower	20.3	E	0.339	0.068	0.271	0.767			
	31.2		0.515	0.095	0.420	0.774			
	39.1		0.648	0.117	0.531	0.775	0.787	0.0188	2.4
	15.0	DMF	0.269	0.058	0.211	0.808			
	29.7		0.518	0.100	0.418	0.810			
	45.7		0.758	0.130	0.628	0.790			
fish meal	21.3	E	0.331	0.090	0.241	0.650			
	40.3		0.641	0.180	0.461	0.657			
	61.7		0.974	0.280	0.694	0.646	0.653	0.00596	0.9
	20.6	DMF	0.339	0.105	0.234	0.653			
	30.6		0.508	0.155	0.353	0.663			
	42.0		0.678	0.202	0.476	0.651			
meat meal	21.2	E	0.216	0.090	0.126	0.341			
	40.2		0.415	0.180	0.235	0.336			
	59.8		0.594	0.246	0.348	0.334	0.333	0.00532	1.6
	20.7	DMF	0.209	0.092	0.117	0.325			
	31.1		0.323	0.144	0.179	0.331			
	41.2		0.429	0.190	0.239	0.333			

Table V. Comparison of the Tryptophan Content of Some Feedstuffs, Determined by the Proposed Method, with Previously Published Values[a] or with Gas Chromatographic Measurements[b]

name	crude protein: N × 6.25	Trp content: g/100 g crude protein measd by acid ninhydrin method	lit. values[a]
soybean meal (extracted)	49.50	1.26	1.26–1.59
soybean meal (crude)	34.65	1.51	1.37
wheat	11.42	1.20	0.8–1.2
maize	10.80	0.885	0.666–1.01
barley	9.00	1.43	1.46–1.50
rye	8.25	0.991	1.11
sunflower	43.25	1.82	1.03–1.46
fish meal	63.7	1.025	0.82–0.96
meat meal	59.5	0.560	0.40–0.64
			GC[b]
lysozyme		6.97	6.94
α-chymotrypsin		6.87	6.86
bovin serum albumin 1		0.726	0.707
human γ-globulin		2.69	2.48

[a] Feedstuffs ingredient analysis table, 1984 edition. [b] Measured as *N,O,(S)*-trifluoroacetyl isobutyl ester in hydrolysates prepared with 6 mol of hydrochloric acid (145 °C, 4 h) in the presence of 3-(2-aminoethyl)indole (tryptamine) (Fábián et al., 1989).

Table VI. Recovery of the Tryptophan Content of Various Ingredients of Diets[a]

sample	amt, mg	A at 380 nm			g Trp/100 g dry sample		Δ rel %
		A	B	A − B	measd	calcd[b]	
diet 1	22.1	0.151	0.049	0.102	0.265		−0.8
	30.6	0.207	0.065	0.142	0.266	0.267	−0.4
	50.5	0.333	0.097	0.236	0.269		+0.8
diet 2	20.2	0.122	0.045	0.077	0.219		±0.0
	41.5	0.230	0.070	0.160	0.222	0.219	+1.4
	62.0	0.339	0.101	0.238	0.221		+0.9
diet 3	17.0	0.123	0.040	0.083	0.281		+0.4
	31.8	0.204	0.050	0.154	0.278	0.280	−1.4
	41.8	0.277	0.072	0.205	0.282		+0.7
diet 4	21.6	0.142	0.032	0.110	0.293		+1.7
	30.6	0.200	0.046	0.154	0.289	0.288	+0.3
	41.3	0.260	0.052	0.208	0.289		+0.3
	61.5	0.393	0.087	0.306	0.286		−0.7

ingredient	composition of diet,[d] % (w/w)							
	diet 1		diet 2		diet 3		diet 4	
	Ing	Trp	Ing	Trp	Ing	Trp	Ing	Trp
soybean meal (extracted)	35.20	0.220			21.96	0.1374	21.96	0.1374
soybean meal (crude)			32.82	0.1713				
maize	49.00	0.0468	50.27	0.0481	47.73	0.0456	47.72	0.0456
sunflower 1[c]					12.36	0.0973		
sunflower 2 (0.0851 g Trp/100 g)							12.36	0.1050
premix (inorganic)	15.20		16.91		18.13		17.96	
total		0.2668		0.2194		0.2803		0.2880

[a] Composition of diets percent (w/w). [b] The total tryptophan content of diet ingredients detailed in the footnote of this table. [c] Sunflower 1 = sunflower, as in Tables II and IV. [d] Ingredient = Ing; tryptophan = Trp.

Table VII. Decrease of the Tryptophan Content of Soybean Meal (Extracted) as a Result of Various Denaturation Procedures[a]

procedure	amt, mg	A − B	g Trp/100 g dry matter		dec,[b] %
			single	mean	
untreated	30.4	0.331	0.626	0.625	(100)
	29.6	0.321	0.624		
HCl + H_3PO_4 (pH ~1.8), neutralized	30.7	0.269	0.504	0.506	81
	30.6	0.270	0.508		
HCl + H_3PO_4 (pH ~2.0), neutralized	29.7	0.255	0.494	0.493	79
	30.8	0.264	0.493		
microwave, 100 °C, 5 min	41.2	0.283	0.395	0.394	63
	40.2	0.275	0.393		
HCl (pH ~2), without neutralization	41.2	0.257	0.358	0.317	57
	41.3	0.255	0.355		

[a] More detailed data: Dévényi et al., 1981. [b] Expressed in the percentage of the untreated sample.

In summary, on the basis of the above detailed data, as well as on the analysis of hundreds of ingredients and diets, the acid ninhydrin method, in its improved form, is an exact and fast possibility for the reproducible estimation of tryptophan in intact proteins.

To further explore the field of tryptophan analyses, we will take part in an interlaboratory study concerning the tryptophan assay of food and feedstuffs. Because of its considerable practical value, we hope to automatize the acid ninhydrin test.

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Investigation of Contents of Some Elements in Soil and Apricots by Atomic Absorption Spectrometry

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In this work, the relationship between the concentrations of six metals (Cd, Zn, Mn, Pd, Cu, Fe) in acetic acid extracts (2.5%) of soil and their respective concentrations in apricots grown in the same soil has been investigated. Soil extracts and apricots have been analyzed by atomic absorption spectrometry with injection or loop sample introduction. Metals were preconcentrated with activated carbon by using ammonium pyrrolidinedithiocarbamate (APDC) as a complexing agent at pH 6.

It is well-known that sodium, potassium, calcium, magnesium, and iron are essential for human life and are supplied by feeding. Improvements in analytical techniques show that copper, chromium, cobalt, manganese, selenium, molybdenum, and zinc are also essential for human life and take part in enzyme structure. It is also well-known that mercury, cadmium, lead, and talium, which are toxic to human life, are also taken through feeding. Many countries accept threshold levels for such elements.

For the analysis of food samples, the main problem is preparation of samples for measurement. Food samples can be decomposed either by wet digestion or by dry-ashing methods, depending on the elements being analyzed. For volatile elements like Cd, Zn, Tl, etc., the wet digestion method is preferred (Stoeppler and Brandt, 1979; Verloo, 1982; Hoenig and Borger, 1983; Demir et al., 1985).

A wide range of extractants have been used in soil analysis to suit many purposes. For agriculture, the total trace-element content of a soil has become less important than the extractable contents. The selective chemical extraction of soil components with various extractants has been outlined (Pickering, 1981).

In soil testing, it is important to select a solvent that can extract many elements simultaneously. Some trace elements in the extractants may be in lower concentrations; thus, many elements cannot be directly determined. In these cases, preconcentration steps and/or different sampling techniques have been applied (Pederson et al., 1980; Roberts et al., 1976; Berndt and Messerschmidt, 1982).

When soil is polluted with toxic elements, there is no possibility of elimination but such a situation may be improved by controlling the soil pH by liming, thus leading to irreversible fixation.

EXPERIMENTAL SECTION

Reagents. All chemicals were analytical reagent grade unless otherwise stated. Deionized water was used throughout. Stock solutions (1000 mg/L) were used to prepare standard solutions of metals by diluting with 2 M HNO_3. Ammonium pyrrolidinedithiocarbamate solution and activated carbon suspension were prepared as stated elsewhere (Gücer and Demir, 1987). Buffer (pH 6) was prepared by mixing 5 mL of glacial acetic acid with 117 g of ammonium acetate and diluting the resultant mixture to exactly 1 L.

Apparatus. A Perkin-Elmer Model 400 atomic absorption spectrometer (without background corrector) assembled with a 5-cm air/acetylene burner head was used for atomic absorption measurements. Single-element hollow cathode lamps (S/C Juniper) were used as a radiation source. Wavelengths were selected from the instrumentation manual, and a 2.0-nm slit width was used. Acetylene and air flow rates of 2.6 and 9.6 L/min, respectively, were used throughout the measurements. Analyses of Fe, Cu, and Mn have been done by a conventional flame atomic absorption spectrophotometric method in which 100-μL sample solutions are aspirated directly into the flame. A loop device (Berndt and Messerschmidt, 1979, 1982) was used as a sample introducer (10 μL) for measurements of Zn, Cd, and Pb. When the loop device was used, a silica tube (6-mm i.d. and 47-mm length) was used as a collector and positioned ca. 5 cm above the burner head. A 10-μL sample solution was injected on the platinum loop and dried by passing 3-A ac current through the loop (ca. 20 s). Then, the loop was transported under the window of collection tube through which the light was passing, and shock heating was applied for 0.2 s elec-

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