lated by using mice as the test animal. The performance of mice improved when buffalo gourd protein diets were supplemented with methionine (Thompson et al., 1978). Favorable performance of mice on diets containing defatted buffalo gourd seed meal suggested a lack of endogenous antinutritional compounds in this material. However, a quantitative determination of nutritional antagonists was necessary prior to their use as a protein source. In this study, the levels of antinutritional factors found in the seed of arid-adapted cucurbit species, particularly buffalo gourd, were similar to those in domesticated cucurbits and were comparable to or less than those in soybean. Evidence proffered in this study strengthens previous data indicating the suitability of buffalo gourd seed protein for incorporation in the diets of monogastric animals. If xerophytic cucurbits are to be grown extensively as crops, there appears to be little concern in the use of whole seed, seed meal, and/or seed protein fractions as food sources.

Registry No. Trypsin inhibitor, 9035-81-8; phytate, 83-86-3; sucrose, 57-50-1; raffinose, 512-69-6; stachyose, 470-55-3.

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Chromium in Kale, Wheat, and Eggs: Intrinsic Labeling and Bioavailability to Rats

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Retention of ^{51}Cr was measured in rats fed 3-g test meals containing 63% sucrose, 10% corn oil, and 27% of a test food radiolabeled intrinsically or extrinsically. The test foods were egg yolk, kale, and wheat radiolabeled intrinsically and egg yolk, kale, wheat, and casein radiolabeled extrinsically. Five-week-old male rats were fed a Cr-deficient semisynthetic diet for 2 weeks prior to and 9 days following the 3-g test meals containing ^{51}Cr-labeled foods. By day 9, only 1.1–2.3% of the ^{51}Cr in the test meal remained in the animal. Retention of ^{51}Cr from casein (2.3%) was not significantly different from retention from egg yolk (1.7%) but was significantly higher than that from kale (1.1%) and from wheat (1.5%). These differences may have reflected dietary Cr content rather than a difference in form. There were no significant differences in the retention of ^{51}Cr due to method of labeling ($p \leq 0.05$). Preparation of foods intrinsically labeled with ^{51}Cr was difficult because of a root–shoot barrier in the case of plants and poor absorption or tissue retention by animals. Several approaches to endogenous labeling were attempted. An aqueous extract of kale subjected to gel permeation chromatography showed a low molecular weight chromium complex similar to that found in alfalfa (M_r 2900).

INTRODUCTION

Although chromium has been recognized as an essential trace element and appears to function in glucose utilization (Gurson and Saner, 1971), its physiological function remains unclear. Deficiency symptoms of glucose intolerance, neuropathy, weight loss, and metabolic encephalopathy occurring in patients receiving long-term parental nutrition were reversed upon supplementation with chromium (Jeejeebhoy et al., 1977; Freund et al., 1979). The recommended safe and adequate range of dietary chromium is 50–200 μg/day, and a daily infusion of 15–20 μg of chromium for patients on total parental nutrition has been proposed (Howard and Michalek, 1984).

Little is known about the bioavailability of chromium from foods. Oral Cr^{3+} is poorly absorbed (<1%) and appears mostly in the feces, regardless of the dose and dietary

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source of chromium (Doisy et al., 1976). Hexavalent chromium is more readily absorbed (Mackenzie et al., 1959) but it is not commonly found in foods. It is likely that any chromate present in the diet is reduced in the gastrointestinal tract from 6+ to 3+ valence (Mertz, 1969). It has been suggested that an organic complex of chromium, soluble in 50% ethanol and termed glucose tolerance factor (GTF), would be better absorbed than an inorganic form (Roginski et al., 1971; Hambridge, 1974; Casey and Hambridge, 1980). Using in vitro oxidation of glucose by rat epididymal fat tissue as a measure of GTF, Toepfer et al. (1973) found brewer's yeast, liver, and wheat grain to be good sources of GTF. However, Haylock et al. (1983) separated chromium from a GTF-active factor isolated from yeast without loss of biological activity. The authors concluded that GTF in brewer's yeast is not a chromium complex. Starich and Blincoe (1982) have characterized an anionic, low molecular weight (M_r 2900), water-soluble chromium complex from alfalfa that is distinct from GTF.

The purpose of this study was to investigate the incorporation of ^{51}Cr into kale, wheat, and eggs and to compare the absorption and retention of ^{51}Cr from these intrinsically labeled foods with extrinsically labeled kale, wheat, eggs, and casein in rats. To better predict which forms of chromium are more available for absorption, we attempted to determine the solubility and molecular weight of the chromium complexes in plants.

MATERIALS AND METHODS

Preparation of Radiolabeled Foodstuffs. To determine the form of chromium most readily taken up by plants, wheat (*Triticum aestivum* L. (Inia 66)) and kale (*Brassica oleracea* (Dwarf Gr. Curled)) were germinated in vermiculite and seedlings were transplanted into 2-L pots containing a modified Hoagland–Arnon nutrient solution (Hoagland, 1950). After 1 week, 2 mg of Cr/pot per week as $CrK(SO_4)_2$ or $CrCl_3$ was added for a total of 4 weeks for kale and 8 weeks for wheat. Some wheat plants were stem injected with 0.34 mg of Cr or 0.68 mg of Cr as $CrCl_3$ or $CrK(SO_4)_2$.

To produce intrinsically labeled wheat and kale, plants were germinated in growing blocks and grown in a circulating hydroponicum system described previously (Levine et al., 1982). A modified Hoagland–Arnon nutrient solution was used (Hoagland, 1950). $^{51}CrCl_3$ (132.3 μCi/μg of Cr in 0.1 M HCl) was added to the nutrient solution (2 μCi of ^{51}Cr/L) at 5 weeks of age of kale for 4 weeks and 1–2 weeks after flowering of the wheat until senescence. In order to produce endogenously labeled seeds of higher specific activity, individial wheat plants were stem injected with 1–30 μCi of $^{51}CrCl_3$ 1–2 weeks after flowering. For stem injection, one needle containing the radiolabel was inserted into the peduncle 2 cm below the head and a second needle at the bottom of the peduncle to act as an air vent. Stem-injected wheat was used in the rat-feeding study. Harvested kale was washed, frozen, lyophilized, and reground into a powder on a Sunbeam blender (Belwood, IL). Wheat kernels were ground into flour on a Wiley Mill.

To obtain labeled eggs, one leghorn hen was gavaged with 0.112 mCi of $^{51}CrCl_3$ for 12 days. The hen was also injected (ip) with an aqueous solution containing 0.464 mCi of $^{51}CrCl_3$ adjusted to pH 5. Eggs were collected for an additional 9 days and hard-boiled for the removal of the yolks. Egg yolks were lyophilized and ground with a mortar and pestle.

Extrinsic labeling of kale, wheat, egg yolk, and casein was accomplished by mixing with $^{51}CrCl_3$, drying at 55 °C, and grinding with a mortar and pestle prior to preparation of the test meal.

Table I. Chromium Content of Test Meals

test meals[a]	content, ng of Cr/g	test meals[a]	content, ng of Cr/g
kale (I)	280	egg (I)	21
kale (E)	250	egg (E)	38
wheat (I)	135	casein (E)	80
wheat (E)	90		

[a] Intrinsically labeled (I) or extrinsically labeled (E).

Rat Feeding Study. Weanling, male Sprague–Dawley rats (250 ± 12 g) were housed individually in stainless-steel cages. For 2 weeks prior to administration of the test meal and following the test meal, they were maintained on an AIN-76 semipurified diet (AIN, 1977) deficient in chromium (80 ng/g) in an attempt to improve absorption. Distilled water was given ad libitum. Rats were randomly divided into groups of six and were meal trained for 5 days prior to the test meal. Rats were fasted for 8 h prior to receiving a 3-g test meal containing 27% of the chromium source (kale, wheat, egg, casein), 63% sucrose, and 10% corn oil. Radiolabeled and nonlabeled sources of chromium were added to achieve a uniform dose of 8000 cpm of ^{51}Cr (50% counter efficiency) among test meals. The chromium concentration for each of the test meals is given in Table I.

Rats were allowed 3 h to consume the test meal. The radioactivity in the rats was assayed by using the counting window of 0.220–0.500 meV in a small animal whole-body counter previously described (Meyer et al., 1983). The rats were subsequently counted on days 0.5, 2.5, 3.25, 4.25, and 9. Whole-body retention of ^{51}Cr was corrected for isotope decay, counter fluctuations, and background. Mean percent retentions of ^{51}Cr were compared by one-way analysis of variance using Duncan's multiple-range test at an α level of 0.05 (Duncan, 1955).

Investigations of Chromium Complexes. Solubility of ^{51}Cr in 80% boiling ethanol and 22 °C or boiling deionized water and 0.01 M phosphate buffer (nonsequentially) from freeze-dried kale and ground whole wheat was determined. An equal amount of food material and aqueous or organic solvent were mixed in a vortex mixer and centrifuged for 30 min at 20000g. The supernatant from seven successive extractious were collected and counted for radioactivity.

The approximate molecular weight of the chromium complex in kale was determined by gel filtration chromatography (Biogel P-6) of an aqueous extract using a 1.5 × 30 cm column. Fractions (1 mL) were eluted at 1 mL/min with a 0.01 M phosphate buffer (pH 7.0) at 4 °C and monitored for ^{51}Cr.

RESULTS

Accumulation of Chromium by Plants. Chromium was added as $CrCl_3$ or $CrK(SO_4)_2$ to the nutrient solution of hydroponically grown wheat and kale or by stem injection to wheat in an attempt to increase the chromium content of these foods. The chromium content of the edible portions of these plants is given in Tables II and III. The control wheat seeds grown without addition of chromium to the nutrient solution contained 70 ng/g. The reported range for chromium content of Australian wheat is 4–98 ng/g (Jones and Buckley, 1977). Chromium content of one wheat sample analyzed in the U.S. was 280 ng/g (Toepfer et al., 1973). Chromium in the form of $CrCl_3$ but not $CrK(SO_4)_2$ added to the nutrient solution increased the chromium content of wheat seeds. Much greater increases in chromium content of the seeds resulted from stem injecting the wheat. Similar increases in chromium content were observed for either form of chromium at both

Table II. Chromium Content of Wheat Kernels Grown Hydroponically with Added $CrCl_3$ or $CrK(SO_4)_2$ or Stem Injected with Two Doses of $CrCl_3$ or $CrK(SO_4)_2$ Plus Grown in Either $CrCl_3$ or $CrK(SO_4)_2$

treatment	content, ng of Cr/g (ambient moisture)
control	70
$CrCl_3$ hydroponic	220
$CrK(SO_4)_2$ hydroponic	43
$CrCl_3$ hydroponic + 0.34 mg of $CrCl_3$ stem injected	1900
$CrCl_3$ hydroponic + 0.68 mg of $CrCl_3$ stem injected	2900
$CrK(SO_4)_2$ hydroponic + 0.34 mg of $CrK(SO_4)_2$ stem injected	2000
$CrK(SO_4)_2$ hydroponic + 0.68 mg of $CrK(SO_4)_2$ stem injected	2300

Table III. Chromium Content and Yield (Leaf and Stem, Fresh Weight Basis) of Kale Plants Grown on Hydroponic Pot Culture Treated with 2 mg of Cr/Pot per Week for 4 Weeks[a]

treatment	moisture, %	content,[b] ng of Cr/g of dry mat	yield, g/plant
control	82	100	19.9 ± 3.1
$+CrCl_3$	77	170	38.0 ± 22.9
$+CrK(SO_4)_2$	78	130	54.0 ± 20.8

[a] n = 8 plants. [b] Leaf and midrib were analyzed. Reference material NBS citrus leaves (SRM 1572): certified, 800 ± 200 ng of Cr/g; found, 830 ± 56 ng of Cr/g.

Table IV. Radioactivity Recovered in Wheat Kernels of Plants Stem Injected with $^{51}CrCl_3$ at Eight Doses and Grown in a Circulating Hydroponicum

dose, μCi of ^{51}Cr	recd radioact cpm of ^{51}Cr/head	recd radioact cpm of ^{51}Cr/g
1	2066	2198
2	15654	19568
5	18137	16193
10	22932	19600
15	25676	27908
20	22000	26506
25	27164	29850
30	32785	27320

Table V. Radioactivity and Yield of Wheat Kernels Grown in a Circulating Hydroponicum with or without Added $^{51}CrCl_3$[a]

treatment	yield, g/head	radioact, cpm/g
control	0.99 ± 0.15	
$+^{51}CrCl_3$	1.04 ± 0.28	122

[a] n = 10 plants.

dosage levels. Accumulation of ^{51}Cr by wheat seeds was also much greater when $^{51}CrCl_3$ was administered by stem injection (Table IV) than through the nutrient solution (Table V). Increasing doses of ^{51}Cr by stem injection resulted in increases in seed ^{51}Cr up to injection levels of

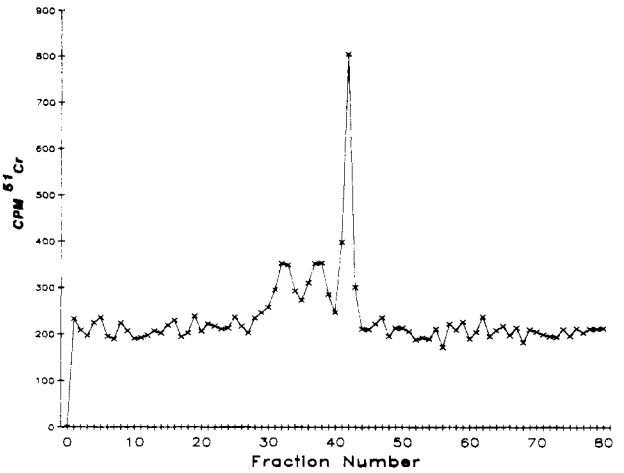

Figure 1. Gel filtration chromatography of kale extracts on Biogel P-6.

15 μCi of ^{51}Cr. Only stem-injected wheat had sufficient ^{51}Cr concentration for subsequent studies. The ^{51}Cr concentration of kale used in the rat feeding study was 14000 cpm/g.

Yields of wheat were not decreased by the presence of chromium except for wheat stem injected with $CrK(SO_4)_2$ at a level of 0.68 mg of Cr (0.32 g/head vs. 0.95 g/head for wheat stem injected with 0.34 mg of Cr as $CrK(SO_4)2$). Although weights of kale increased with addition of chromium, chromium concentration was comparable among treatments (Table III).

Accumulation of ^{51}Cr by Eggs. Small amounts of ^{51}Cr were accumulated in eggs after gavaging the hen daily for 12 days with $^{51}CrCl_3$: 394 cpm/g of egg yolk, 103 cpm/g of egg white, 621 cpm/g of egg shell. Intraperitoneal injection was required to produce sufficient concentration of ^{51}Cr in eggs for the rat feeding study: 29225 cpm/g of egg yolk, 177 cpm/g of egg white, 305 cpm/g of egg shell.

Rat Feeding Experiment. Retention curves of ^{51}Cr by rats from intrinsically labeled wheat, kale, and eggs and extrinsically labeled wheat, kale, eggs, and casein appear in Table VI. After 9 days, only 1.1–2.3% of the dose remained from all ^{51}Cr sources. Whole-body retention of ^{51}Cr from casein extrinsically labeled with $^{51}CrCl_3$ (2.3 ± 0.7%) was not significantly different from intrinsically labeled eggs (1.7 ± 0.4%) but was significantly higher than in groups receiving intrinsically labeled kale (1.1 ± 0.4%) or wheat (1.5 ± 0.7%). Retention of ^{51}Cr from extrinsically labeled eggs (1.9 ± 0.5%), kale (1.3 ± 0.6%), and wheat (1.4 ± 0.4%) was not significantly different from their intrinsically labeled counterparts.

Solubility of Chromium from Kale and Wheat. Most of the ^{15}Cr from kale could be extracted in water, whereas less than one-fourth of wheat ^{51}Cr could be extracted in room temperature or boiling water (Table VII). ^{51}Cr did not exist in an ethanol-soluble complex.

Table VI. Percent Retention of ^{51}Cr from Intrinsically and Extrinsically Labeled Kale, Wheat, Egg, and Casein by Rats

treatment[a]	% retention 0.5 day	2.5 days	3.25 days	4.25 days	5.25 days	9.0[b] days
kale (I)	67.0 ± 5.7	11.2 ± 2.1	6.6 ± 1.9	3.5 ± 0.5	2.9 ± 0.5	1.1 ± 0.4[a]
kale (E)	59.5 ± 14.7	6.0 ± 1.2	3.7 ± 0.3	2.8 ± 0.5	2.4 ± 0.5	1.3 ± 0.6[a,b]
wheat (I)	82.7 ± 13.0	9.7 ± 5.7	4.7 ± 1.7	2.9 ± 0.8	2.7 ± 0.3	1.5 ± 0.7[a,b]
wheat (E)	70.3 ± 13.3	7.6 ± 2.2	4.0 ± 0.6	2.5 ± 0.6	2.4 ± 0.4	1.4 ± 0.4[a,b]
egg (I)	83.2 ± 15.6	10.2 ± 1.7	5.2 ± 0.9	2.8 ± 0.5	2.4 ± 0.4	1.7 ± 0.4[a,b,c]
egg (E)	81.7 ± 12.9	8.3 ± 3.8	3.2 ± 1.0	1.9 ± 0.8	1.9 ± 0.4	1.9 ± 0.5[b,c]
casein (E)	81.1 ± 11.5	7.0 ± 4.1	3.2 ± 1.5	3.2 ± 0.7	3.4 ± 1.1	2.3 ± 0.7[c]

[a] Intrinsically radiolabeled (I) or extrinsically radiolabeled (E). [b] Values not followed by the same superscript are significantly different from each other ($p < 0.05$).

Table VII. Percent of ^{51}Cr Extracted by Various Solvents from Wheat and Kale

solvent	% ^{51}Cr extracted	
	wheat grain	kale
water, 22 °C	23	87
water, 100 °C	21	
boiling 80% ethanol	1	
0.01 M phosphate buffer	4	

Molecular Weight of Kale Chromium Complex. Gel filtration chromatography of an aqueous extract of kale resulted in a large ^{51}Cr peak at fraction number 42 with smaller peaks at fraction numbers 33 and 38 (Figure 1). Using identical chromatography conditions, Starich and Blincoe (1983) observed ^{51}Cr peaks from alfalfa extracts at fraction numbers 42 and 52. These fractions were later determined to differ by the presence of an amine group (Starich and Blincoe, 1983). Using Biogel P-G and standards, they estimated the molecular weight of the two ^{51}Cr containing peaks as 1000 and 600. However, analysis of the same alfalfa extract on Sephadex G-25 and G-50 resulted in a single peak of ^{51}Cr at molecular weight 2900.

DISCUSSION

Chromium was poorly accumulated by all biological systems studied. A pilot study for the purpose of screening plants for their ability to accumulate chromium revealed that the edible tissues of cereals (wheat) and legumes (bush beans and soybeans) accumulated less chromium than green, leafy vegetables (kale). As predicted from the pilot study, hydroponic kale could be produced with sufficient ^{51}Cr for a rat feeding study by adding ^{51}Cr to the nutrient solution. However, wheat grain did not accumulate sufficient ^{51}Cr unless ^{51}Cr was administered by stem injection. This may not result in the physiologically natural form of chromium. Similarly, Cary et al. (1977) reported leaves of a number of plants accumulated more chromium than other plant parts when $KCrO_4$ was added to soil. Most of the ^{51}Cr administered to nutrient solutions of hydroponically grown wheat, corn, potatoes, tomatoes, peas, kidney and mung beans, cauliflower, beetroot, radishes, carrots, lettuce, and barley remained in the roots and was poorly translocated to the tops (Cary et al., 1977; Lahouti and Peterson, 1979). Stem injection allowed the root and shoot barrier to be bypassed in the present study. Interestingly, accumulation of ^{51}Cr by grain of stem-injected plants appeared to plateau after 15 μCi of ^{51}Cr.

Chromium content generally increases with increasing application via hydroponic solution up to levels toxic to plants (Schmitt and Weaver, 1984). Chromium toxicity is typically manifested by decreased yields that occur at levels as low as 0.5 ppm in hydroponically grown soybeans (Turner and Rust, 1971). However, small amounts of chromium have been reported to increase plant yields (Haas and Brusca, 1961; Mertz, 1969). Chromium supplied at 1 ppm as $CrCl_3$ or $CrK(SO_4)_2$ enhanced yields of kale (Table III). Wheat, stem injected with $CrK(SO_4)_2$ at 0.68 mg of Cr, was the only treatment resulting in a marked decrease in grain yield.

Oral dosing of a hen with $^{51}CrCl_3$ did not result in appreciable ^{51}Cr accumulation in eggs, which suggests chromium was poorly absorbed by the hen. When the gastrointestinal tract was avoided by giving an intraperitoneal injection of $^{51}CrCl_3$, sufficient ^{51}Cr accumulated in the egg yolks for the subsequent rat feeding study. Whether deposition of ^{51}Cr into the eggs following injection vs. oral administration occurs in the same form is uncertain.

The rat also did not absorb and retain much ^{51}Cr from any of the labeled foods (Table VI). Retention of ^{51}Cr from animal sources was slightly greater than from plant sources. Although absorption of Cr has been reported to be independent of dose (Mertz et al., 1965), it should be pointed out that total Cr content was higher in the plant sources. However, extrinsic and intrinsic labeling produced similar results in spite of differences in Cr content between extrinsically and intrinsically labeled sources. Visek et al. (1959) observed an absorption less than 0.5% in rats receiving an oral dose of $^{51}CrCl_3$. Huffman and Allaway (1973) observed a retention of less than 0.5% in rats receiving radiochromium from either CrO_2^{2-} or bean leaf after 48 h. Mertz and Roginski (1971) reported that an amino acid–chromium complex (glucose tolerance factor) is absorbed more readily than inorganic chromium. If it can be assumed that ^{51}Cr from $^{51}CrCl_3$ equilibrated with the foods that were extrinsically labeled in the present study, as has been proposed for alfalfa (Blincoe and Starich, 1983), then all of the foods tested most probably contained organic Cr complexes. Whether the 1.1–2.3% retention of ^{51}Cr from these foods would have been higher than for inorganic ^{51}Cr salts administered by gavage is not certain. Due to the low concentrations of ^{51}Cr in intrinsically labeled foods fed in the test meals and subsequent low retention of ingested ^{51}Cr at day 9, ^{51}Cr activity approached background counts per minute of ^{51}Cr. An average rat background ^{51}Cr was determined on rats of similar size to those used in the study. Results may be slightly overestimated since background counts per minute were not determined on each rat prior to administration of the test meal. Also, more precise results may have been achieved if test meals could have been prepared with higher concentrations of ^{51}Cr.

The low molecular weight ^{51}Cr complex in an aqueous extract from the intrinsically labeled kale may be the same ^{51}Cr complex extracted from alfalfa (Starich and Blincoe, 1982) and bean leaves (Huffman and Allaway, 1973). The water-soluble ^{51}Cr complex in alfalfa was stable throughout the gastrointestinal tract of rats (Starich and Blincoe, 1983). Approximately 30% of the alfalfa ^{51}Cr was available to the rat when retention was adjusted for endogenous turnover. However, net retention of alfalfa ^{51}Cr was similar to that for kale reported in the present study (Blincoe, personal communication).

Gel filtration chromatography was not performed on wheat and eggs because of the small amount of labeled materials produced. The ethanol-insoluble nature of the ^{51}Cr complex in wheat contrasts with the results of others who found 49% of wheat ^{51}Cr extractable in boiling 80% ethanol (Huffman and Allaway, 1973) and 37.8% of wheat chromium extractable in 50% ethanol (Toepfer et al., 1973). Toepfer et al. (1973) reported that egg yolk is a concentrated source of chromium, over 50% of which is extractable in 50% ethanol. However, the ability of egg yolk chromium to potentiate insulin-stimulated in vitro oxidation of glucose was half that of wheat.

The results reported here suggest that chromium in kale exists as a low molecular weight, water-soluble complex similar to that described for alfalfa, crested wheat grass, and bean leaves (Starich and Blincoe, 1983; Huffman and Allaway, 1973). Accumulation of chromium by seeds is largely blocked at the root–shoot barrier, and absorption of chromium by chickens and rats is largely blocked at the gut. Perhaps the limited absorption of chromium by plants and animals lessens the risk of chromium toxicity but may contribute to marginal chromium deficiency.

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Continuous Spectrophotometric Assay for Plant Pectin Methyl Esterase

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A simple continuous assay for plant pectin methyl esterase (EC 3.1.1.11) has been developed. During the enzymatic reaction, the galacturonic acid methyl esters in pectin are hydrolyzed, producing acid. In the assay, the pH indicator bromothymol blue is protonated to produce a change in the absorbance at 620 nm. The absorbance change is spectrophotometrically monitored. The assay is calibrated with galacturonic acid. The sensitivity of the assay is 0.020 μmol of acid produced/min, and the precision is 12% (relative standard deviation). The assay requires only 2–3 min and 2 mL of substrate/trial. The measured activities are directly proportional to the amount of commercial pectin methyl esterase added, and the activity obeys saturation kinetics. In addition, the assay can be used to determine pectin methyl esterase in crude extracts of plant tissues. There is no interference from colored components or other activities in the extracts.

INTRODUCTION

Pectin degradation plays an important role in plant disease (Cooper, 1983), fruit ripening (Hobson et al., 1984), nutrition (Oppermann et al., 1973), and food product stability (McLellan et al., 1985). For example, ripening fruit softens because pectin and other cell wall carbohydrates are broken down enzymatically. Pectin, which is composed of α-1,4-linked galacturonic acid and galacturonic acid methyl ester, is degraded by a group of pectinases (Rexova-Benkova and Markovic, 1976). The galacturonic acid methyl esters are hydrolyzed by pectin methyl esterase (PME) (EC 3.1.1.11) (reaction 1).

$$\text{pectin–COOCH}_3 + \text{H}_2\text{O} \rightarrow \text{pectin–COO}^- + \text{H}^+ + \text{CH}_3\text{OH} \quad (1)$$

The existing assays for pectin methyl esterase are inconvenient or insensitive. Several methods have been described for determining the products of pectin hydrolysis. For example, the methanol produced (reaction 1) can be determined chromatographically (McFeeters and Armstrong, 1984) or colorimetrically (Wood and Siddiqui, 1971). The acid produced (reaction 1) can be determined by titration with a pH stat or a pH meter (Kertesz, 1937; Lee and Macmillan, 1968). The chromatographic method for methanol determination is very sensitive but is not convenient for routine enzyme determination. The colorimetric method for methanol and the titration methods require large volumes of reactants and are time consuming. In an alternative assay, hydrolysis of *p*-nitrophenyl acetate by the esterase is measured spectrophotometrically (Huggins and Lapides, 1947). This method is not useful for determining PME in crude plant extracts because it is neither specific nor sensitive.

We have developed a new continuous assay for PME that is convenient, sensitive, and specific. The new assay, like several qualitative assays previously described for PME (Rexova-Benkova and Markovic, 1976; Zimmerman, 1978), is based on the color change of a pH indicator during the PME-catalyzed reaction. As the ester bonds are hydrolyzed, acid groups are produced (reaction 1) and the

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