

and heptachlor are not expected to fluoresce, as no conjugated double bonds are present in their structures. DDT-type insecticides do not fluoresce because of their halogen-substituted ring structure, and the same is apparently true for the chlorinated phenoxyacetic acid materials. Rotenone contains a dimethoxyphenyl group, but this apparently does not contribute to fluorescence in the solvent systems used. Methoxychlor, in which two methoxyphenyl groups are present, is also inactive. Aramite, in which electron donation through hyperconjugation in the *p*-*tert*-butylphenoxy nucleus is not possible, also fails to fluoresce, as does Diazinon, which contains a pyrimidine nucleus.

In the low concentration range (below 1 γ per ml.), fluorescence is directly proportional to concentration; at higher concentrations self-quenching becomes

significant and may lead to low results.

For comparative purposes the absorbance of piperonyl butoxide at λ_{max} 288 using a Beckman Model DU and 1-cm. silica cells was determined. At a concentration of 1 γ per ml., this absorbance was 0.027 unit. The sensitivity using the spectrofluorometer is 0.01 γ per ml.

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ISOTOPE-LABELED FERTILIZERS

Preparation of Radioactive Polynutrient Fertilizers Having Specified Phosphate Solubilities

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Several series of radioactive mixed fertilizers, having different degrees of water solubility, were prepared in a small-scale production plant for use in greenhouse and field investigations. The mixtures were classified as mixed-salt types, prepared by the blending of suitably chosen inorganic salts, and commercial types, derived from the treatment of phosphate rock with sulfuric or nitric acid. High phosphate availability and close control of the phosphate water solubility were obtained in the mixed-salt types. More difficulty was encountered in regulation of solubility and availability in the commercial types.

THE RADIOISOTOPE of phosphorus, phosphorus-32, is commonly employed as a tracer in agronomic investigations of soil, plant, and fertilizer reactions. Since 1947, the Fertilizer Investigations Research Branch, as a result of an agreement with the Atomic Energy Commission, has been engaged in the small-scale production of fertilizers and fertilizer materials containing this tracer element (3). The labeled products are shipped to a number of universities and state or federal cooperating agencies.

The type of material produced has been determined by the needs and desires of the using groups. Recently, attention has been focused on polynutrient combinations in which the water solubility of the contained phosphate falls within defined limits. Field and greenhouse investigations are being conducted at several locations on the influence of the degree of this solubility and on the effects of granulation to various particle sizes. A summary of the information

gained by experience in the preparation of these materials, including a description of known factors which influence variability in products, is presented.

Manufacturing Methods

The labeled polynutrient fertilizer mixtures were classified as: mixed-salt types, prepared by the blending of suitably chosen inorganic salts, and commercial types, derived from superphosphate or from nitric acid treatment of phosphate rock (nitric phosphate). All of these materials were prepared by a slurry process. Certain of the manufacturing operations, such as labeling, blending and drying of mixtures, and granulation of pulverulent products, are common to the preparation of both types and will be described separately.

Labeling. Radioactive phosphorus is obtained from the Oak Ridge National Laboratory as an aqueous solution of potassium dihydrogen phosphate of

negligible nonorthophosphate content. In the fertilizer manufacture, portions of this solution are added to each phosphate source to supply a specific activity in the product of approximately 0.15 mc. per gram of phosphoric oxide.

Radiation intensities normally encountered in the manufacture of radioactive fertilizers are such that precautionary safeguards against exposure and particulate contamination must be strictly observed. Protective clothing, special equipment and tools, and adequate shielding and ventilation are necessary (3). All of the processing equipment, such as grainers, grinders, granulators, and screeners, are mounted in hooded enclosures; chemical reactions, such as acidification, ammoniation, precipitation, and filtration, are carried out in a hood equipped with facilities for the collection of waste materials.

Blending and Drying. These operations are carried out in a grainer, a specially designed mixer-evaporator,

which consists of a stainless steel container mounted in a water bath and equipped with an agitator which scrapes the side walls and bottom. Infrared lamps and blowers hasten the evaporation and drying. Desired proportions of the components are combined in the grainer in an aqueous slurry and the mixture is heated to 95° to 100° C. and agitated for several hours while the water is evaporated. The dried product is ground in a hammer mill. Particle size of the ground product is approximately 90% through a 40-mesh screen; 75% through 100 mesh.

In this slurry technique, intimate contact of components is achieved, accompanied by formation of stable salts through ionic interchange. The material is hence a fine-grained mixture (2) that possesses considerably greater uniformity and stability than is obtainable with the use of conventional dry-mixing procedures.

Granulation. A major advantage of granulated fertilizers as opposed to pulverulent goods is the improved physical quality of the materials on long-term storage. However, materials labeled with phosphorus-32 must be placed in the soil as soon as possible after manufacture because of relatively rapid decay of the radioactive isotope. Consequently, granulation was provided only in cases in which the agronomic influence of granulation is to be studied. In recent years, somewhat less than 10% of the tagged fertilizers shipped from this section was in granulated form, chiefly in polynutrient mixtures.

In order to effect granulation, the pulverulent material from the blending and drying operation is subjected to a water spray as it is rotated in a horizontal stainless steel drum. The granules are then dried and sized to specifications by mechanical sieving.

Preparation of Mixed-Salt Fertilizers. Several series of mixed-salt-type fertilizers of varying water-soluble phosphate content were prepared. Nitrogen, phosphorus, and potassium are supplied by suitable inorganic salts such as ammonium nitrate, ammonium sulfate, monoammonium phosphate, dicalcium phosphate, and potassium chloride. The details for the preparation of this type of mixture vary with the specifications for the finished fertilizer. Water solubility of the phosphate, for example, is regulated by suitable proportionment between monoammonium and dicalcium phosphates.

Dicalcium phosphate is synthesized by the dropwise addition of a 50% phosphoric acid solution to a lime slurry. The reaction is carried out at room temperature with continuous agitation. The resulting slurry is digested for about 1 hour at 95° to 100° C. The precipitate is separated by decantation, washed with water, then acetone, dried, and

screened. In radioactive preparations, the acid is tagged with phosphorus-32 prior to addition to the lime. To produce the desired mixtures, the dicalcium phosphate is slurried in hot water in the grainer, and nitrogen and potassium salts are added as hot saturated solutions—generally followed by the addition of diatomaceous earth as a filler. This filler aids in the granulation of soluble salt mixtures and has negligible agronomic effects. The mixture is blended and dried in the manner previously described.

When monoammonium phosphate is the phosphate source, it is tagged with phosphorus-32 after dissolution in the minimum quantity of hot water. This solution is added to the grainer and the preparation of the desired mixture is carried out in the usual manner. In mixtures containing both dicalcium and monoammonium phosphate, each is prepared as a tagged material and introduced into the grainer prior to addition of the other components.

Preparation of Superphosphate-Containing Mixtures. The first step is the preparation of a superphosphate, which is subsequently ammoniated and/or incorporated with nitrogen and potassium salts to form the final mixture. Phosphate rock, to which phosphorus-32 has been previously added in radioactive preparations, is placed in a mixer and a 70% sulfuric acid solution is added rapidly, with agitation. The reaction is allowed to proceed until gas evolution is virtually complete. The batches are cured in a recirculating air oven at 65° to 70° C. for 1 to 3 days, depending on their size. The product is shaved out of the container and disintegrated to a particle size of approximately -14 mesh.

For the ammoniation, the superphosphate is slurried in water at room temperature for about 30 minutes, and a commercial aqua ammonia solution (28% ammonia) is added dropwise in an amount sufficient to reach the desired stage of neutralization (4). The reactants are agitated and maintained at room temperature. To prepare the mixed fertilizer, the nitrogen and potassium salts in hot saturated solutions and diatomaceous earth are added to the ammoniated superphosphate and mixed; the water is evaporated in the usual manner.

Preparation of Nitric Phosphates. Several series of products of graduated phosphate solubility have been prepared which include a nitric phosphate as the source of water-insoluble phosphate. This nitric phosphate is synthesized by the slow acidulation of phosphate rock at room temperature with a stoichiometric quantity of 50% nitric acid solution. A small amount of magnesium hydroxide is added to the rock prior to acidulation

in order to achieve consistency of water-soluble phosphate content. After dissolution of the rock, phosphoric acid is added to adjust the calcium oxide-phosphoric oxide mole ratio to 2.5 to 1. A labeling solution of radiophosphorus is next incorporated, and the mixture is slowly ammoniated at room temperature. An ammonium nitrate solution is finally added to adjust the nitrogen-phosphoric oxide weight ratio to 1 to 1. This mixture is evaporated to dryness in a grainer and pulverized.

This nitric phosphate is employed in the series as the member of lowest solubility. Proportioned quantities of labeled monoammonium phosphate solution are added to nitric phosphate slurries just after the ammoniation step to produce series members of higher solubility. The completely soluble member contains no nitric phosphate, as it is derived wholly from the ammonium salt.

Composition of Polynutrient Mixtures

Methods of Analysis. Total phosphorus is determined on intermediate materials to be used as ingredients of mixtures, and on all finished products. The official volumetric method for fertilizers (7, 2.14) is used. Official methods for the determination of water-soluble phosphorus (7, 2.16), available phosphorus (7, 2.19), and total nitrogen (7, 2.28) also are employed in the analysis of polynutrient mixtures. Potassium is determined with the use of a flame photometer after preparation of a solution of the sample by an official method (7, 2.40a). Free water is estimated by measurement of the weight loss of two grams of sample when placed in a desiccator over anhydrous magnesium perchlorate for 3 hours at atmospheric pressure.

6-12-12 Fertilizers. Several large groups of 6-12-12 mixtures have been prepared in connection with a comprehensive agronomic investigation. Results of analyses of these materials afford an opportunity for comparative study of the influence of manufacturing procedure on the composition of the fertilizer, inasmuch as the materials were prepared to specific water solubilities and included both radioactive and nonradioactive preparations of both mixed-salt and commercial types in pulverulent and in granulated forms.

The composition of the mixed-salt fertilizers is given in Table I. Ingredients of the mixtures included dicalcium phosphate, monoammonium phosphate, ammonium sulfate, potassium chloride, and diatomaceous earth, except that no monoammonium phosphate was added in formulating materials at the 0 to 10% solubility level. The

Table I. Composition of Mixed-Salt 6-12-12 Fertilizers

Lot No.	Production Period	Physical Form	Total P ₂ O ₅ , %	Water-Soluble P ₂ O ₅ , % of Total P ₂ O ₅		Available P ₂ O ₅ , % of Total P ₂ O ₅	Total N, %	Total K ₂ O, %	Free H ₂ O, %
				Intended range	Found				
Nonradioactive Mixtures									
N 191	I	Pulverulent	11.9	25-30	31.3	...	5.7
		Granulated ^a	12.2		30.9	...	5.5
N 192	I	Pulverulent	11.9	50-60	60.5	...	5.8
		Granulated	12.0		61.0	...	5.8
N 195	I	Pulverulent	11.9	0-10	10.6	...	5.7
		Granulated	12.5		10.6	...	5.7
Radioactive Mixtures									
R 296	II	Pulverulent	11.7	0-10	9.0	99.7	5.7	12.2	1.9
		Granulated	12.3		8.0	99.6	5.9	11.9	0.3
R 297	II	Pulverulent	11.2	50-60	61.1	99.5	5.7	...	3.8
		Granulated	11.9		59.4	99.7	5.8	...	0.4
R 304	III	Pulverulent	11.0	0-10	9.0	99.7	5.5	12.1	2.9
		Granulated	12.0		7.7	98.8	5.6	11.7	...
R 305	III	Pulverulent	11.5	50-60	58.4	99.6	5.7
		Granulated	11.8		57.1	99.7	5.7	...	2.8
R 311	IV	Pulverulent	11.4	0-10	10.7	99.5	5.6	...	2.1
		Granulated	12.2		10.1	99.4	5.8	...	0.2
R 312	IV	Pulverulent	11.4	50-60	57.7	99.1	5.5	11.4	2.6
		Granulated	12.5		57.2	98.8	5.9	11.7	0.4

^a All 6-12-12 granules are 8-20 mesh.

water-soluble phosphate content was in every instance within or slightly above the intended range. The somewhat high solubilities of materials at the 25 to 30% and 50 to 60% levels were occasioned by an overestimation of the proportion of monoammonium phosphate in the products in the first production period, brought about by insufficient allowance for the enhancement of the solubility of dicalcium phosphate by ammonium sulfate (5). The surplus of soluble phosphate was carried along in subsequent production periods for the sake of reproducibility.

The content of individual nutrients in the mixtures generally fell below formula requirement as a consequence of residual moisture in the finished fertilizer. However, the ratio of total nitrogen to total phosphoric oxide closely approximated the desired 1 to 2.

Granulation resulted in higher-analysis mixtures, chiefly because of the more efficient drying treatment to which the granules were subjected. The lower

water content of the granulated materials is indicated in the last column of the table. Preferential agglomeration of some soluble salts during granulation also contributed to variation in composition of the different fractions.

Only a negligible portion of the phosphate was not available in these mixed-salt assemblages, and procedural variations occasioned by the handling of radioactive material were not a serious factor in reproducibility of the finished fertilizers (Table I).

In the commercial-type 6-12-12 mixtures, the phosphate was supplied as ordinary superphosphate and the nitrogen by ammoniation of the superphosphate supplemented with ammonium nitrate or ammonium sulfate. Water solubility of the phosphate was controlled for the most part by the extent of neutralization with ammonia (4).

As it is difficult to obtain a product containing phosphate of low water solubility simply by the ammoniation of superphosphate, two alkaline supple-

ments were included in the preparation of the mixtures at the 0 to 10% solubility level. Magnesium hydroxide was incorporated with the phosphate rock before acidulation to inhibit the effect of fluorine; calcium hydroxide was added to the mixture after ammoniation, for the purpose of changing any ammonium phosphates that may have formed to dicalcium phosphate.

The chemical composition of the superphosphates from which the 6-12-12 commercial type mixtures were derived is shown in Table II and that of the mixtures in Table III. The addition of magnesia, which amounted to approximately 1% as magnesium oxide in the final product, in the acidulation mixture resulted in a drop in both water-soluble and available phosphorus content of the superphosphates. Satisfactory reproducibility of superphosphate composition was obtained; the six lots having no added magnesia contained an average of 20.9% phosphoric oxide, of which 84% was water-soluble and 90% was available.

The difficulty of the regulation of water solubility by control of the extent of neutralization with ammonia is pointed up by the results in columns 6 and 7 of Table III. The products of the over-all reaction with ammonia varied with the ambient conditions in the mixture, which included the character of the superphosphate and local ammonia concentration, among other things, and consequently, the nature of the water-insoluble phosphate in the ammoniated superphosphate was subject to considerable uncontrolled variability. Hence, the low water solubilities found in the mixtures at the 50 to 60% level seem to be the result of overammoniation. The

Table II. Composition of Ordinary Superphosphates Used in 6-12-12 Commercial-Type Mixtures

Lot No.	Total P ₂ O ₅ , %	Water-Soluble P ₂ O ₅ , % of Total P ₂ O ₅	Available P ₂ O ₅ , % of Total P ₂ O ₅
R 294	19.4	90.3	95.5
N 198	21.4	79.9	87.5
R 302	20.9	83.9	91.5
N 200A	20.5	83.8	89.5
R 309	21.6	81.8	89.0
N 202	21.5	82.3	89.6
R 295 ^a	20.2	71.8	83.4
R 303 ^a	20.9	70.1	80.3
R 310 ^a	20.5	71.7	80.8

^a Contains added magnesium hydroxide.

Table III. Composition of Commercial-Type 6-12-12 Fertilizers Derived from Superphosphate

Lot No.	Production Period	Lot No. of Parent Superphosphate ^a	Physical Form	Total P ₂ O ₅ , %	Water-Soluble P ₂ O ₅ , % of Total P ₂ O ₅		Available P ₂ O ₅ , % of Total P ₂ O ₅	Total N, %	Total K ₂ O, %	Free H ₂ O, %
					Intended range	Found				
Nonradioactive Mixtures										
N 199	II	N 198	Pulverulent	10.9	25-30	30.6	87.7	5.4	11.3	6.2
			Granulated	11.8		25.3	87.5	5.8	12.0	...
N 201	III	N 200A	Pulverulent	11.7	25-30	30.7	86.3	5.5	11.7	...
			Granulated	12.2		25.2	83.7	5.8	12.0	...
N 203	IV	N 202	Pulverulent	12.0	25-30	30.6	88.3	5.7	11.2	...
			Granulated	12.2		26.3	88.3	5.7	11.2	...
Radioactive Mixtures										
R 298	II	R 295	Pulverulent	12.1	0-10	4.3	81.9	5.8	...	2.7
			Granulated	11.9		4.9	83.0	5.9	...	1.1
R 299	II	R 294	Pulverulent	12.1	50-60	50.8	95.3	5.9	12.3	0.9
			Granulated	11.9		49.9	95.7	6.0	12.0	1.4
R 306	III	R 303	Pulverulent	11.6	0-10	6.3	78.8	6.9	...	2.2
			Granulated	11.8		6.1	79.4	7.0	...	0.9
R 307	III	R 302	Pulverulent	11.5	50-60	32.6	89.3	5.8	11.8	2.2
			Granulated	11.6		30.5	89.3	5.8	11.6	...
R 313	IV	R 310	Pulverulent	12.1	0-10	3.7	79.0	6.1
			Granulated	12.5		4.5	79.5	6.4	...	1.3
R 314	IV	R 309	Pulverulent	12.1	50-60	27.3	86.9	6.0	11.7	2.1
			Granulated	12.2		27.4	87.5	6.1	11.9	...

^a For composition, see Table II.

low availabilities (column 8) at the 0 to 10% level might be ascribed to the use of magnesia in the superphosphate.

Granulated 12-12-12 Mixed-Salt Fertilizers. Several groups of 12-12-12 mixtures of varying degrees of water-soluble phosphate content have been prepared. Inasmuch as at least a portion of each series was granulated, analysis of these materials affords an opportunity to show the magnitude of variation of nutrient content among the different fractions of these granular materials.

A pronounced variability in composition is indicated in the results for one typical group of products in Table IV. Here, six mixtures containing phosphate of different degrees of water solubility were prepared in pulverulent and three granulated particle sizes. Solubility of the phosphate was again varied by interchange of the carriers dicalcium phosphate and monoammonium phosphate. The 28- to 48-mesh fraction was regularly low in phosphate regardless of the source of the nutrient, whereas the coarser 4- to 6-mesh size usually contained an excess. Despite this bias, control of water solubility was satisfactory throughout the sieve range.

A clear indication of the influence of granulation on the nitrogen-phosphoric oxide ratio is given in another group of 12-12-12 mixtures, shown in Table V. In the 4- to 6-mesh granules, there was a deficiency of nitrogen as well as a surplus phosphate content, a combination seemingly brought about by segregation of components during granulation. In the finer 28- to 48-mesh fraction, on the other hand, the two nutrient elements were much closer to the proper 1 to 1

ratio and to the desired formulation. The nitrogen-phosphoric oxide ratio averaged 0.91 in the coarse fraction and 0.98 in the finer cut. Water solubility of the phosphate generally fell within the intended range in both particle sizes. Potassium salts apparently were not segregated in these slurry mixtures to the extent usually found in dry mixes.

Nitric Phosphates. The chemical content of five groups of tagged nitric

phosphates is shown in Table VI. The materials are listed in chronological order of manufacture, each series requiring approximately 1 week for completion. As the more soluble materials were prepared chiefly from pure salts, their solubility regulation was precise. Differences between intended solubilities and those actually obtained were greater in the lower solubility range, a direct result of the preponderance of ammoni-

Table IV. Total and Water-Soluble Phosphorus in Sized Portions of Labeled 12-12-12 Fertilizers of Mixed-Salt Type

Lot No.	Granule Size, Mesh	Total P ₂ O ₅ , %	Water-Soluble P ₂ O ₅ , % of Total P ₂ O ₅	
			Intended range	Found
R 51	4-6	12.2	0-3	1.9
	14-20	12.0	0-3	1.9
	28-48	11.3	0-3	0.9
	Pulverulent ^a	12.4	0-3	2.0
R 52	4-6	12.6	8-10	...
	14-20	12.3	8-10	...
	28-48	11.6	8-10	...
	Pulverulent	12.3	8-10	9.3
R 53	4-6	12.8	18-20	...
	14-20	12.5	18-20	...
	28-48	12.2	18-20	21.7
	Pulverulent	12.6	18-20	19.7
R 54	4-6	12.8	28-30	27.8
	14-20	12.5	28-30	28.8
	28-48	11.5	28-30	...
	Pulverulent	12.6	28-30	30.6
R 55	4-6	12.2	45-50	...
	14-20	12.3	45-50	...
	28-48	11.4	45-50	45.2
	Pulverulent	12.6	45-50	40.4
R 56	4-6	12.2	85-90	85.2
	14-20	12.0	85-90	88.8
	28-48	12.0	85-90	...
	Pulverulent	12.6	85-90	82.1

^a Substantially -40-mesh.

ated, rock-acid mixtures as the phosphate source. Water solubilities in these cases were increased by the probable formation in the ammoniation step

of ammonium phosphates (2) and to some extent by the influence of soluble salts present on the solubility of the dicalcium phosphate contained in the

mixture. In each series, the total content of phosphate and of nitrogen increased directly with the solubility. This is an expected consequence of the formulation and the decision to avoid the use of filling material in any of the mixtures.

Phosphate availability varied inversely with the proportion of phosphate derived from the rock—presumably, a consequence of the use of magnesia in the acidulation step. This magnesia supplementation may hence be considered a means of compromise between high availability and compositional reproducibility.

The content and quality of phosphate in one series of simulated nitric phosphates that was prepared solely from pure salts is given in Table VII. Dicalcium phosphate prepared from lime and phosphoric acid was used as the source of water-insoluble phosphate instead of the product of ammoniation of rock-acid solution. Materials prepared in this manner are the most satisfactory from the standpoint of high availability and controlled water solubility.

Other Formulations. In order to fulfill specific requirements of projected agronomic experiments, mixtures of both mixed-salt and commercial types have been prepared to formulations other than those previously mentioned. Several series of 12-12-0 materials of controlled water-soluble phosphate content were produced from dicalcium phosphate, monoammonium phosphate, ammonium nitrate, and a filler. In another study, the two phosphates were paired with such salts as the sulfates of potassium, calcium, magnesium, aluminum, and sodium; and with potassium chloride, ammonium sulfate and nitrate, calcium nitrate and carbonate, and urea. Additionally, a group of 10-10-0 and 10-10-10 mixtures of high availability was synthesized with the use of an enriched superphosphate. In all these cases, the previously described manufacturing methods sufficed to produce the desired materials.

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Table V. Composition of Labeled Granular 12-12-12 Fertilizers of Mixed-Salt Type

Lot No.	Granule Size, Mesh	Total N, %	Total P ₂ O ₅ , %	N/P ₂ O ₅ , Wt. Ratio	Water-Soluble P ₂ O ₅ , % of Total P ₂ O ₅		Total K ₂ O, %
					Intended range	Found	
R 266	4-6	11.4	12.6	0.90	0-3	1.8	11.7
	28-48	11.9	12.1	0.98	0-3	1.5	11.7
R 267	4-6	11.3	12.4	0.91	8-10	9.0	...
	28-48	12.1	12.2	0.99	8-10	9.1	...
R 268	4-6	11.0	12.7	0.87	18-20	18.0	11.7
	28-48	12.1	12.6	0.96	18-20	18.2	11.9
R 269	4-6	11.7	13.0	0.90	28-30	28.2	12.2
	28-48	12.0	12.1	0.99	28-30	29.6	11.8
R 270	4-6	10.9	12.0	0.91	40-45	41.2	12.0
	28-48	12.0	12.0	1.00	40-45	41.9	12.1
R 271	4-6	10.8	11.3	0.96	85-90	81.6	...
	28-48	11.5	12.1	0.95	85-90	81.6	...

Table VI. Composition of Labeled Nitric Phosphates (Commercial Type) of Varying Water Solubility

Lot No.	Total P ₂ O ₅ , %	Water-Soluble P ₂ O ₅ , % of Total P ₂ O ₅		Available P ₂ O ₅ , % of Total P ₂ O ₅	Total N, %	Free H ₂ O, %
		Intended	Found			
R 166	20.5	10	14.7	75.4	17.6	3.2
R 167	21.2	25	32.0	89.8	19.2	...
R 168	21.1	35	41.3	91.6	20.3	...
R 169	22.3	55	56.9	93.7	21.2	...
R 170	22.9	70	69.7	95.2	22.0	...
R 171	25.4	100	100	100	25.2	...
R 173	20.3	10	11.3	78.9	17.4	...
R 174	21.0	25	31.8	88.7	19.2	...
R 175	21.3	35	39.8	92.0	...	1.9
R 176	22.0	55	57.0	94.1
R 177	22.9	70	65.2
R 178	25.9	100	100	...	25.3	...
R 182	20.4	10	15.1	...	17.7	...
R 183	21.1	25	34.8	90.5	19.5	...
R 184	21.3	35	42.3	90.6	20.3	...
R 185	22.0	55	59.0	93.4	20.9	1.6
R 186	22.5	70	73.8	...	22.4	...
R 187	26.3	100	100	...	25.3	...
R 257	20.0	10	11.5	...	17.4	...
R 258	21.0	25	30.7	...	19.5	...
R 259	21.6	35	40.0	...	20.3	...
R 260	22.3	55	59.4	...	21.1	...
R 261	23.2	70	71.7	...	22.0	2.2
R 262	26.8	100	97.0	...	24.8	...
R 279	20.3	10	11.9	80.3	17.5	...
R 280	21.5	25	30.9	...	19.3	3.5
R 281	20.4	35	35.1	...	18.1	3.7
R 282	22.9	55	59.3	...	21.6	...
R 283	23.1	70	70.6	...	21.7	...
R 284	26.1	100	100	...	25.4	...

Table VII. Total and Soluble Phosphorus in Labeled Nitric Phosphates of Mixed-Salt Type

Lot No.	Total P ₂ O ₅ , %	Water-Soluble P ₂ O ₅ , % of Total P ₂ O ₅		Available P ₂ O ₅ , % of Total P ₂ O ₅
		Intended	Found	
R 198	21.4	0	2.7	>99.5
R 199	21.4	15	16.5	>99.5
R 200	23.3	35	34.3	>99.5
R 201	25.3	42	46.6	>99.5
R 202	24.6	59	61.1	>99.5
R 203	25.7	74	76.9	>99.5
R 204	25.5	100	100	>99.5

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ENZYME ASSAY

Quantitative Determination of Peroxidase in Sweet Corn

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A procedure is described for the quantitative determination of peroxidase in whole-kernel sweet corn. The method involves the colorimetric determination of the color formed when the enzyme oxidizes orthophenylenediamine in the presence of hydrogen peroxide. Starch is removed from a buffered extract of corn by alcohol precipitation and centrifugation. The absorption curve for the oxidized dye shows a maximum absorbance at 430 m μ under the conditions used in this assay. The color formed from the peroxidase-catalyzed oxidation of the orthophenylenediamine follows the Beer-Lambert law if a correction term is applied for the nonenzymatic oxidation of the dye. The assay is independent of the sample weight used for color development. Enzyme activity was determined in corn which had been steam blanched for various periods from 0 to 8 minutes. Enzyme activity decreased with increasing blanch time.

DATA—mostly qualitative—on the destruction of peroxidase in many vegetables may be found in the literature (2, 4, 8). No thermal destruction data for sweet corn peroxidase are available, as no satisfactory quantitative procedure has been worked out for its determination. This study was undertaken in an effort to develop such a method.

Although the most resistant microorganisms may be destroyed in a matter of seconds as processing temperatures approach 300° F. (3), certain enzymes are not completely and irreversibly inactivated by such a thermal process (5). Peroxidase has been particularly noted for having more heat resistance than bacterial spores at high temperatures. Storage of processed foods containing this enzyme may lead to quality deterioration.

The destruction of peroxidase in processed foods has been studied to determine the relationship between this enzyme and off-flavor development during storage. A "viny" off-flavor developed in canned peas processed under high-temperature, short-time conditions when peroxidase was not completely and irreversibly destroyed (5). Labbee and Esselen (7) reported that off-flavors developed in fresh-pack pickles when peroxidase was not completely destroyed during the pasteurizing operation. Dietrich and coworkers (7) reported that blanched frozen peas containing active

peroxidase received flavor scores lower than those for samples containing no enzyme after storage at -10° F. Their finding agreed with those of Joslyn (6), in which he found that peroxidase activity paralleled the formation of off-flavors.

Most of the common methods of estimating peroxidase activity are colorimetric. Guyer and Holmquist (5) and Farkas, Goldblith, and Proctor (4) used variations of the guaiacol oxidation procedure. However, this procedure cannot be used for determining small concentrations of peroxidase in sweet corn, as the large amount of corn required to give sufficient enzyme for adequate color development results in starch turbidity which prevents direct reading in the spectrophotometer.

Wallerstein and associates (10) developed a fluorophotometric procedure for the quantitative determination of peroxidase in potato tubers using orthophenylenediamine as substrate. Reddi, Esselen, and Fellers (9) determined the amount of peroxidase in apple tissue also using *o*-phenylenediamine as substrate. They stopped the reaction after 5 minutes by adding 2 ml. of saturated solution of sodium bisulfite to 15 ml. of reaction mixture. The color was extracted with ethyl acetate and its absorbance measured in an Evelyn photoelectric colorimeter using filter No. 420.

Because the product formed by the

peroxidase-catalyzed oxidation of *o*-phenylenediamine was found to be relatively stable, this method was believed to be usable for the quantitative determination of small amounts of peroxidase in sweet corn.

Recommended Procedure

Approximately 4 grams of fresh whole-kernel corn is made to a total volume of 250 ml. with pH 6.5 phosphate-citrate buffer and is blended for 3 minutes in an Osterizer. The buffer is prepared by combining 14.2 parts of 0.2*M* disodium hydrogen phosphate and 5.8 parts of 0.1*M* citric acid. Ten milliliters of this extract is diluted to 250 ml. with buffer. A 25-ml. aliquot of the blended and diluted sample is transferred to a 250-ml. centrifuge bottle. Another 25-ml. aliquot of each sample is taken for a colorimeter blank reading. The sample must be mixed thoroughly before the aliquot is taken, because much of the enzyme is located in solid particles which tend to settle on standing. The samples are placed in a constant temperature bath at 25° C. for 30 minutes.

To the sample, in which the color is to be formed, the following reagents are added: 1 ml. of 1% *o*-phenylenediamine (in 95% ethyl alcohol; fresh every 4 hours) and 1 ml. of 0.3% hydrogen peroxide (in distilled water). The reaction is allowed to proceed for 5 minutes,