procedures yielded values of the same order of magnitude, by statistical criteria, more than half of these were significantly higher by the *O. malhamensis* method than by the *L. leichmannii* method. The *L. leichmannii* method is better suited to routine practice. The data do not preclude the possibility that some hitherto untested type of animal food product might contain sufficient nonspecific active material to invalidate its *L. leichmannii* potency value. In view of this possibility it would be wise to assay by both methods products not previously so tested.

Acknowledgment

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FREEZE-PROCESSING EFFECTS ON RICE

Effect of Freeze-Processing on Amyloclastic Susceptibility, Crystallinity, and Hydration Characteristics of Rice

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This study was initiated to determine if starch retrogradation accompanied freeze-processing of rice and to obtain additional information on changes occurring in rice during processing. Freeze-processing resulted in development of a *B*-type x-ray diffraction pattern, and a lowered β-amylase susceptibility. This resistance was almost completely neutralized by heating an aqueous suspension 45 minutes at 45° C. or higher. The changes in x-ray pattern and amyloclastic susceptibility were similar to those associated with retrogradation. The water-holding capacity of whole-grain freeze-processed rice was much greater than that of either whole-grain raw or cooked unfrozen controls. Where the effects of gross physical structure were minimized by grinding, raw rice (at 80° C. and above) had the greatest water absorption. These studies demonstrated a physical as well as a chemical effect due to freezing.

When cooked rice was frozen under defined conditions and then air-dried, it exhibited an exceptionally rapid rate of water uptake, even at room temperature (*11*). It became chalky, took on a characteristic porous or spongelike texture, and had a greater specific volume than either a raw or an unfrozen control—i.e., one that had been cooked and then dried. It was postulated that this starch, spongelike formation was due to either retrogradation or a closely related mechanism.

Previous investigators (*10*, *13*) reported that measurement of the suceptibility of starch to attack by amylases was valuable in following the effects of physical treatment and "aging" on starch. They concluded that the amyloclastic susceptibility diminished as a result of retrogradation. Katz and van Itallie (*8*) reported that retrograded starch was in a semicrystalline state that could be characterized by a *B*-type x-ray pattern.

The present study was initiated not only to clarify whether retrogradation took place during the freeze-processing of rice, but also to obtain additional information on the changes in rice starch during processing. The measurement of amyloclastic susceptibility and the determination of the degree of crystallinity by x-ray appeared to be the most generally accepted criteria for the detection of retrogradation. However, because hydration characteristics have been used (*6*) to differentiate varieties of rice, it was anticipated that they also would be of value in differentiating rice processed by various methods. Therefore, to demonstrate possible retrogradation and differences in the properties of rice due to processing, the amyloclastic susceptibility, x-ray diffraction pattern, and hydration characteristics of freeze-processed rice were compared to those of an unfrozen control and to raw rice.

Materials and Methods

Raw Materials. Rexoro variety rice, foundation stock, 1957 crop (Rice Pasture Experiment Station, Beaumont, Tex.) was used in these studies. Two aliquots from the same lot of rice were received; one consisted of milled whole grains and the other sample was the original rough or unmilled rice. Those parts of the milled and the rough rice which were not required for immediate experimentation were stored in sealed containers at 4° C. The rough rice, kept as a reserve supply in case additional material was required, was used about 10 months after it had been put into storage. It was milled by the standard methods (*12*) using the McGill sheller and huller immediately before the experiment.

Sample Preparation. Two basic processed products, cooked rice and freeze-processed rice, were studied along with the original raw milled rice. In preparing the processed products, a portion of the milled rice was cooked in an excess of boiling distilled water until done (about 20 minutes), then it was drained and washed on a 20-mesh screen. Half of it was spread in a thin layer to dry under ambient conditions. The dried product was called "cooked rice." The other half was sealed into two-piece friction-top cans (401 × 411) and held for 24 hours in a cabinet freezer at −22° ± 3° C. After freezing, the cans were transferred to a refrigerator at 4° ± 1° C. for 48 hours, the rice was then removed from the cans and dried in the same manner. The resultant product was designated as "freeze-processed" rice.

These samples were used for all phases of this investigation, except for the amylograph studies. The greater portions of these products were ground to pass a 40-mesh screen of a Wiley mill; however, the whole grains were used for some of the hydration measurements.

A similar set of products was prepared from another portion of the milled rice. These were processed in the same manner, except that the drying was accomplished by lyophilization instead of by ambient air. The rice was placed in 2-liter flasks, filling them to about one fourth capacity, and freezing was accomplished by twirling the flasks in a mixture of acetone and dry ice until the contents were solidly frozen. Drying was carried out for about 36 to 48 hours at a pressure of about 50 microns. The lyophilized material was ground and used for some of the β-amylase susceptibility tests and for the x-ray diffraction studies.

For the amylograph studies, raw, cooked, and freeze-processed rice were prepared from the rough rice. Because of the large amounts required for these determinations, a larger capacity No. 1 Wiley mill was used for grinding, employing a 0.5-mm. screen (ca. 35-mesh).

Hydration Measurements. The hydration rate of whole-grain rice was measured at various temperatures, from 65° to 100° C., by modifying a previously described method (*11*). Briefly, 5.00-gram samples were used with 40 ml. of water, and after exactly 45 minutes at the desired temperature, the rice was separated from the excess water (containing the soluble and insoluble solids) on a 30-mesh stainless steel screen. The total solids loss was determined by collecting the filtrate in tared beakers and drying to constant weight for 16 hours in a forced air oven at 104° ± 2° C. The wet weight of the hydrated rice divided by the initial dry weight, corrected for total solids lost, was used as the "hydration index."

The amount of water uptake on ground, rather than on whole-grain rice, was determined by a modified swelling power method (*9*). The rice samples (1.000 gram each) were placed in 125-ml. glass-stoppered Erlenmeyer flasks, and 25 ml. of distilled water were added. The flasks were placed in a constant temperature bath (rectangular manometric apparatus, American Instrument Co., Silver Spring, Md.) set at the desired temperature between 65° and 95° C. They were continuously shaken for 45 minutes through a 27-mm. stroke at about 68 cycles per minute. To separate the hydrated rice from the cooking water containing the solubles, the mixture, in 40-ml. conical tubes, was centrifuged at about 2000 × C for 30 minutes. The hydrated rice was weighed after the supernatant liquid had been removed by suction. Soluble solids were estimated by evaporation of the supernatant material. The hydration index was calculated on the same basis as for whole-grain rice.

Determination of Amyloclastic Susceptibility. The amyloclastic susceptibility of the starch in the rice was measured by digestion with one of two amylases (Nutritional Biochemical Corp., Cleveland, Ohio). The α-amylase was of bacterial origin and the β-amylase was from barley. According to the supplier the activities of the enzymes were, respectively, 1800° and 2000° Lintner. For each test 0.250 gram of ground rice was transferred to a 125-ml. glass-stoppered Erlenmeyer flask and 5 ml. of water were added. The flask was then placed in a constant temperature bath. After 2 minutes, 10 ml. of buffer were added followed 4 minutes later by 5 ml. of freshly prepared 0.5% aqueous solution of the enzyme. During hydrolysis the mixture was shaken continuously. β-Amylolysis was allowed to proceed for 20 hours at 30° C. with 0.1*M* acetate buffer, pH 4.8; hydrolysis by α-amylase was carried out from 5 minutes to 6 hours at 65° C., and from 1 to 20 hours at 35° C. using an 0.1*M* phosphate buffer, pH 7.0.

The amount of hydrolysis was determined on a 5-ml. aliquot by a ferricyanide method (*1*). These values are expressed in terms of milligrams of maltose monohydrate per gram dry weight of sample.

Studies were made on the effect of preheating treatments in conjunction with the amyloclastic susceptibility tests. A weight of 0.250-gram of ground rice was suspended in 5 ml. of water and held for 45 minutes in a bath at a constant predetermined temperature, in the range of 30° to 100° C. The preheated samples were then subjected to the action of β-amylase as in the above described methods.

Amylograph Viscosity Measurements. In an attempt to evaluate the amyloclastic susceptibility by another method, the viscosities of mixtures of buffer, rice meal, and amylase were measured, as they were heated at a uniform rate, through the gelatinization range of the starch. A research-type amylograph known as a Viscograph (C. W. Brabender Instruments, Inc., South Hackensack, N. J.) with 700-cm. gram cartridge sensitivity was employed. The procedure used was essentially that of "Cereal Laboratory Methods" (*2*) with the following changes: rice, equivalent to 50 grams dry weight, ground to pass a 0.5-mm. screen was used; McIlvaine's citrate phosphate buffer, pH 4.6, diluted 1 to 4, was substituted; the total weight of sample and buffer was 500 grams. Enzyme concentration was based upon 2 parts per 100 parts dry weight of rice. The viscosity was recorded as the temperature increased from 30° to 90° C., held for 30 minutes at 90° C., lowered to 50° C., and held for 30 minutes at 50° C.

X-Ray Diffraction Methods. The sample was packed into a glass ring, mounted on the back pinhole of the collimator, and then was exposed for 4 hours to nickel-filtered copper radiation (Cu-Kα,λ = 1.5418 A.). The diffraction pattern was recorded on flat film at a 6-cm. film to sample distance. An additional set of x-ray diffraction patterns was obtained on lyophilized as well as on air-dried samples to confirm the data reported here. In this series a 1.5-hour exposure was used with a 5-cm. film distance. The number and the discreteness of the lines in the x-ray diffraction pattern are a measure of "crystallinity."

Results and Discussion

Hydration. The difference in hydration characteristics between whole-grain freeze-processed rice and cooked rice (Figure 1) was at least partially attributable to the physical texture of the freeze-

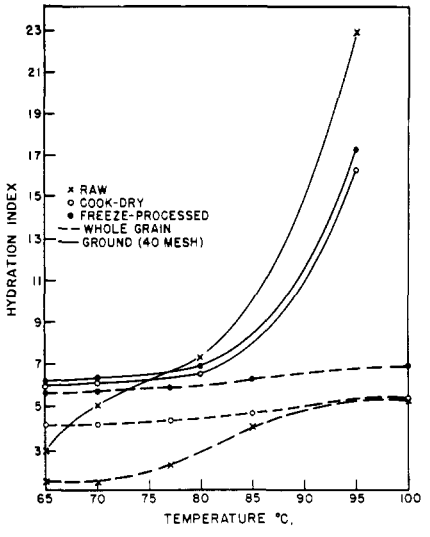

Figure 1. Effect of temperature on hydration indices of whole grain and ground rice preparations

Hydration time 45 minutes

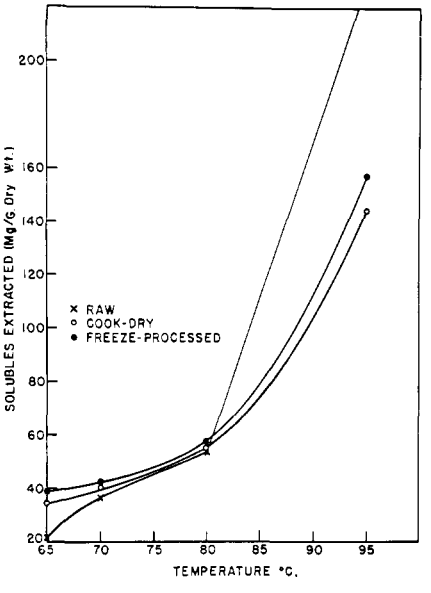

Figure 2. Effect of temperature on extractability of solubles from ground rice preparations

Extraction time 45 minutes, 40-mesh samples

Table I. α-Amylase Susceptibility of Rice Samples, Effect of Hydrolysis Temperature and Preheating

(Hydrolyzed at pH 7.0, 0.1*M* phosphate buffer)

Sample and Treatment	Susceptibility, Mg. Maltose Monohydrate/G. Dry Weight: Hydrolyzed at 65° C. for 2 hr.	Hydrolyzed at 35° C. for 20 hr.
Raw[a]	50	111
[b]	232	462
Cooked[a]	180	422
[b]	218	440
Freeze-processed[a]	208	440
	220	450

[a] No preheat treatment.
[b] Preheated 45 minutes at 100° C.

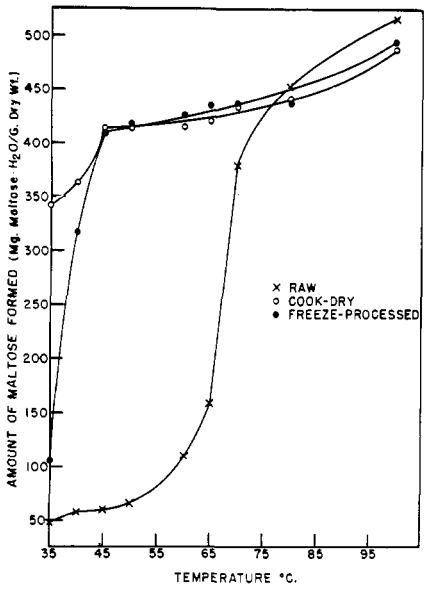

Figure 3. Effect of heating aqueous suspensions of rice preparations on subsequent β-amylase susceptibility

Preheat treatment 45 minutes
Hydrolysis in 0.1*M* acetate buffer, pH 4.8, for 20 hours at 30° C.

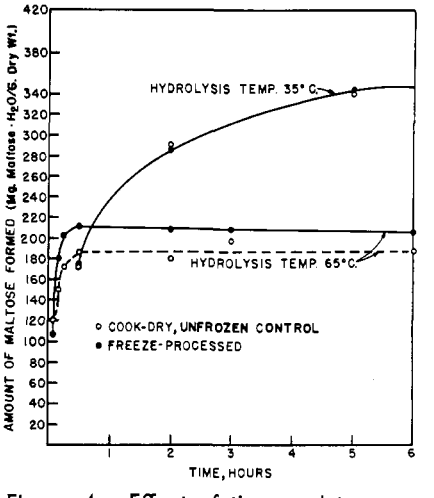

Figure 4. Effect of time and temperature on amount of α-amylase hydrolysis of processed rice samples in 0.1*M* phosphate buffer, pH 7.0

treated material. When hydration was determined on ground rice, rather than on the whole-grain basis, the difference in the amount of hydration between these samples diminished considerably, demonstrating that the effects of porosity on hydration were either minimized or removed by grinding. Raw whole-grain rice has a relatively compact structure which does not inhibit complete gelatinization, but may be a mechanical barrier, especially at temperatures above the gelatinization range, that could prevent complete hydration of the more centrally located starch cells. Thus, when the mechanical restriction is removed by grinding, the amount of hydration in raw rice should and did increase greatly as the temperature was increased.

Measurements of water absorption of ground rice at temperatures below 65° C. are not reported, because erratic results were obtained, especially on the processed samples. This was attributed to the failure of centrifugation to yield stable, compact residues from which most of the free water could be withdrawn. A gradual and progressive increase in the volume of the centrifuged residue occurred. In many instances this volume increased 100% within 10 minutes, immediately after centrifugation. Variation of the time and amount of centrifugal force (up to 30,000 ×C) failed to halt the expansion.

The dry weights of solubles extracted from ground rice and separated by centrifugation were plotted against hydration temperatures (Figure 2). The shape of the curves appeared to be similar to those for hydration. The values obtained for soluble solids probably reflect only the prior removal of solubles from the processed samples.

Amyloclastic Susceptibility. Hydration methods are valuable in determining differences in physical state, as a result of processing, but they may not measure changes in chemical characteristics. Studies with α- and β-amylases, however, demonstrated a specific heat-reversible difference in susceptibility. The behavior of raw and cooked rice toward amylases is in agreement with the established concepts that ungelatinized starch—i.e., raw rice—is resistant to the action of amylases and that gelatinization increases susceptibility. However, freeze-processed rice, which was also gelatinized, was exceedingly resistant to β-amylolysis.

α-Amylase is a liquefying enzyme that attacks the starch molecules simultaneously at various places. It is not capable of splitting α-1,6′-glucosidic linkages, but it can bypass them (*16*). β-Amylase on the other hand, does not break up the molecules, but merely shortens the end chains until the action on α-1,4′ linkages is stopped by branch points or is impeded by secondary valence forces such as those that accompany retrogradation (*4*). Cross linking has been shown to impart increased resistance to amylase activity (*15*). Since the action of α-amylase is random, it evidently can bypass additional links that were formed as a result of retrogradation and therefore it failed to differentiate between the two processed products (Table I). β-Amylase did not attack freeze-processed rice as readily (Figure 3), indicating that other inhibiting linkages were present. Thus, it may be assumed that freezing of cooked rice results in retrogradation of some of the starch which altered its amyloclastic susceptibility.

Preliminary experiments conducted at 65° C. indicated the possibility that the α-amylase had been inactivated. Only

30 mg. of maltose were produced per gram of rice when a 0.5% solution of α-amylase was heated for 30 minutes at 65° C. in 0.1*M* phosphate buffer, pH 7.0, and then mixed with the substrate for 30 minutes. A control that had not been heated prior to reaction with the substrate yielded 159 mg. of maltose per gram. Further proof of enzyme inactivation was obtained when there was no hydrolytic action after the enzyme solution had been heated for 1 hour at 65° C. Subsequent studies with α-amylase were conducted at 35° C. (Figure 4). At this temperature there was a progressive increase, the degree of hydrolysis increasing to 440 mg. of maltose per gram in 20 hours.

To avoid the possibility of retrograding the processed samples by prolonged drying at a low temperature (*14*), lyophilization was used in place of ambient air drying. Cooked rice gave a hydrolysis value of 285 mg. of maltose per gram upon β-amylolysis, and freeze-processed rice gave 52 mg. per gram. Lyophilization increased these values to 439 and 166 mg. per gram, respectively. The greater resistance of the unlyophilized controls indicates that either some additional retrogradation occurred during drying under ambient conditions, or that lyophilization caused an increase in susceptibility.

To differentiate further between freeze-processed and cooked rice, aqueous suspensions were preheated and then subjected to digestion by β-amylase. Soaking freeze-processed rice at 45° C. or higher brought the susceptibility to hydrolysis to about the same level as that for cooked rice. This finding agrees with that reported by Volz and Ramstad (*13*). They observed that reheating at 55° C. for 10 minutes was efficient in restoring the susceptibility of various retrograded starch pastes to the action of Takadiastase. However, at 80° C. and above, the processed rice samples showed less enzymatic susceptibility than the raw rice. This difference in susceptibility may represent the inability to obtain complete restoration of retrograded materials by heating, and is similar to observations reported by Whistler (*15*).

Amylograph Viscosity. An amylograph was also used in an effort to evaluate the susceptibility of the rice samples toward the action of amylases. The viscosity curves (Figure 5) showed the processed samples to be more susceptible than raw rice to the action of β-amylase. The similarity of reaction of the cooked and freeze-processed rice samples to the enzyme excludes the amylograph viscosity measurement as a method for differentiating processed rices. However, there appeared to be some relation between the transition temperatures of processed samples, when run without enzyme, and their amylase susceptibility as obtained by the previous method. Therefore, the determination of the temperatures, where the first rise in viscosity is noted in the amylograph, is worthy of additional study as a more rapid procedure for predicting amylase-susceptibility.

Table II. X-Ray Data of Rice Samples Compared to Those of *A*-, *B*-, *C*-, and *V*-Type Reference Diffraction Patterns[a]

Rice Samples				Reference Values[b]			
Raw	Cooked	Freeze-processed	Freeze processed; dried at 70° C.[c]	A-type	B-type	C-type	V-type
		15.69S	15.78VF		15.77S		
		11.57VF	11.71F			11.6F	
8.84F			9.02VF		8.34VF		
7.79F		7.69VF					
	6.73M		6.77M			7.05M	7.17M
					6.25M		
5.85S		5.93VF	5.92VF	5.79M	5.79M		
		5.14S	5.17S		5.15S		
5.03S	5.09F			5.02S			
4.40F	4.45MS	4.46M	4.45S	4.42M	4.52M	4.5M	4.45S
		3.96F	4.01VF		4.04M		
3.84S				3.76S			
		3.66F	3.64VF		3.69M		
3.33F		3.34VF		3.40F	3.38F		
				2.62VF	2.87F		2.79M

[a] *d* spacings in A. Relative intensities: S, strong; M, medium; F, faint; VF, very faint.
[b] *A*, *B*, and *C* values from Clark (*3*), *V* from Katz (*7*).
[c] Dried at 70° C. forced air oven in place of drying at room temperature.

X-Ray Diffraction. To verify further the presence of retrogradation, as indicated by β-amylase susceptibility, an x-ray diffraction study was made. Starches—when retrograded—assume a crystalline state that typically gives the *B*-type x-ray diffraction pattern (*8*). The *B*-type pattern was not shown (Table II) by either raw or cooked rice; therefore, it must have been caused by freezing. Raw rice has an *A* pattern characteristic of cereal starches, and cooked rice has either one of the *C*-types or a *V*-type diffraction pattern. The *V*-type has been attributed (*5*) to the amylose-fat complex and is identified by a strong 4.4 A. line.

The x-ray diffraction pattern is greatly dependent upon the temperatures used to dry the aqueous starch pastes. Drying above 60° C. will result in an *A*-type pattern (*3*). When freeze-processed rice was dried at 70° C., there was a decrease in crystallinity and the *A*-type became more prominent. This decrease in crystallinity paralleled the β-amyloclastic susceptibility of preheated (at 45° C. or higher) freeze-processed rice. X-ray data from cooked rice that had been lyophilized, rather than ambient air-dried, did not show any evidence of the *B* pattern. This is a further indication that prolonged drying at room temperature resulted in some retrogradation of the cooked rice.

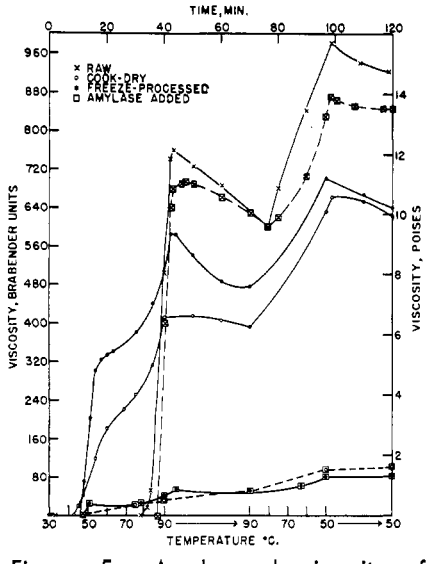

Figure 5. Amylograph viscosity of rice preparations and effect of added β-amylase

Phosphate buffer, pH 4.7, sample to enzyme ratio 50 to 1

Conclusions

These studies have shown that retrogradation was induced by freeze-processing rice. Evidence has also been presented that drying of cooked rice under ambient conditions leads to changes similar to retrogradation. The methods described, especially β-amylolysis, may be valuable in the classification and evaluation of the effects of processing upon the physical and chemical properties of rice.

Acknowledgment

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IRRADIATION EFFECTS IN MEAT

Detection of Amines Produced on Irradiation of Beef

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The volatile bases in beef were studied in an effort to identify the compounds responsible for the characteristic unpleasant odor of beef preserved by irradiation. The beef used in this work received 2.33 and 3.72 megarads of gamma radiation, and its odor was due in part to volatile bases that were measured quantitatively by the Conway-Byrne microdiffusion method and by gas chromatography. The amine fraction of the volatile bases was estimated quantitatively by a colorimetric method and by gas chromatography. Methylamine and ethylamine were identified by gas and paper chromatography as the major components of the amine fraction, with at least four other amines being detected also.

THIS STUDY was undertaken to identify the organic compounds responsible for the characteristic unpleasant odor of beef preserved by gamma radiation. Prior to this work, several groups of workers had found chemical changes that were significant, but amines had not been reported as a cause of the objectionable odor. Batzer and Doty (*2*) found abnormally large amounts of volatile sulfur compounds in irradiated beef. Sribney, Lewis, and Schweigert (*11*) found carbonyl compounds and peroxides. Sharpless, Blair, and Maxwell (*10*) found ethylamine in irradiated aqueous solutions of alanine. Tolbert and Noller (*12*) found that irradiation of dry glycine produced methylamine. Because amines have such powerful unpleasant odors it seemed important to determine whether they are components of the undesirable odor of irradiated beef.

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Experimental Methods

Preparation of Irradiated Beef. The beef used was boneless sirloin butt that had been trimmed of excess fat and ground to ensure thorough mixing. Each batch of meat was packed in No. 2 cans, frozen in dry ice, and shipped to the Materials Testing Reactor, Arco, Idaho, where one third of the cans received an irradiation dose of 3.72 megarads, and another third received 2.33 megarads of gamma radiation from waste fission products. The remaining third of the cans constituted the control samples, receiving identical treatment with regard to shipping and temperature, but no exposure to irradiation. All samples subsequently remained in dry ice until they were opened for examination.

Detection of the Volatile Bases in the Odor. The contribution of the volatile bases to the odor of irradiated meat was appraised by acidifying the condensate obtained from lyophilization of the meat. The condensate from irradiated meat had the usual wet-dog or burnt-hair odor which could be modified considerably by acidification to pH 2 with hydrochloric acid. The acidified condensate from irradiated meat still had a stronger odor than the condensate from unirradiated meat, but much of the recognizable unpleasant odor had been eliminated by the addition of acid.

Measurement of Total Volatile Bases. The Conway-Byrne (*3, 4*) microdiffusion technique was used to measure the total volatile bases in irradiated and unirradiated beef. The moisture in the original meat was determined by the AOAC method (*1*). Samples for the study were taken from two entirely separate batches of meat and from different cans within each batch.

Extracts were prepared by chopping 100 grams of meat with 200 ml. of water for 5 minutes in a Waring Blendor. The resulting mixture was centrifuged and then filtered. The filtrate was diluted with an equal volume of water, and 1.0 ml. of the resulting solution was placed in the Conway-Byrne unit using saturated potassium carbonate solution to liberate the volatile bases. The major