

- (21) Roistacher, C. N., Eaks, I. L., Klotz, L. J., *Plant Disease Repr.* **39**, 202 (1955).
- (22) Roistacher, C. N., Klotz, L. J., Eaks, I. L., *Calif. Agr.* **11** (10), 11-12 (1957).
- (23) Roistacher, C. N., Klotz, L. J., Kolbezen, M. J., Staggs, E. A., *Phytopathology* **47**, 533 (1957).
- (24) Weidel, H., *Ber.* **12**, 1995 (1879).
- (25) Winning, C. W., personal communication.
- (26) Woldman, N. E., *J. Am. Pharm. Assoc.* **18**, 14 (1929).
- (27) Zuffanti, S., *J. Am. Chem. Soc.* **63**, 3123 (1941).

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FUNGICIDES FOR PACKAGED FOODS

Sorption of Ammonia by Fruits, Vegetables, Eggs, and Fiberboard in Dynamic Systems

The comparative rates of sorption and desorption of ammonia gas surrounding 23 kinds of fruits, vegetables, and eggs have been evaluated in a dynamic system with a spectrophotometric technique supplemented by direct chemical assay. Ambient ammonia concentrations ranged from about 50 to 700 p.p.m. in air. Sorption rates varied from about 1 to about 150 p.p.m. per hour. The "buffer" capacity of fiberboard for ammonia has been demonstrated.

THE COMMERCIAL USE of gaseous ammonia to help reduce the fungal decay (19, 20) of citrus fruits during shipment and storage, in commercial cartons, has required evaluations of the comparative rates of sorption and desorption of this gas by the fruits. Roistacher *et al.* (19) gave many of the requirements for successful fumigations with ammonia gas. They stressed that critical minimal concentrations of ammonia surrounding the fruit must be maintained during certain critical periods to prevent germination or to kill the organisms during their early vegetative stages. This is a problem within the fiberboard cartons of citrus fruits, as this in-package environment represents a dynamic system. Ammonia gas quickly establishes unstable sorption-desorption equilibria with carton components. With fruit a more complex equilibrium is established which may include sorption, metabolism, solution, salt formation with acids, and other processes (13, 16). Hydrogen bonding and salt formation are probably involved in the fiberboard equilibrium. Fortunately, for practical purposes both major sorption processes are rapid and dependent upon external ammonia concentration; thus, fiberboard can act as a buffer or a reservoir to assist in maintenance of desired concentrations of ammonia in air surrounding the fruits (19).

Because ammonia gas is fungicidal to a number of mold organisms (17), the ammonia treatment may find practical

applications to other edible commodities. In addition to assisting in the design of a practicable treatment (8), sorption-desorption data help clarify the possible problem of persisting residues of the sorbed ammonia which must be considered under Public Law 518. Under this law and in its applications to control the blue-green molds decay of citrus fruits, for example, ammonia has been declared (5) exempt from the requirement of a tolerance; further, the fruit labeling clause of Section 403K of the Food, Drug, and Cosmetic Act has been waived (14) for this recommended usage, if the container (package) is labeled.

This report is largely concerned with the citrus fruit-ammonia equilibrium and with supplemental clarification of the role of the carton components in this equilibrium. Twenty other fruits and vegetables, and eggs, have been evaluated for comparison with the behavior of citrus fruits.

Ammonia has long been known to occur as a normal constituent of the blood, feces, saliva, semen, tears, and urine of many mammals, including man. It also occurs commonly in most plant tissues, including those of citrus fruits. When ammonia was introduced into plant leaves by vacuum infiltration of organic acid salt solutions, Mothes (15) found it resulted in amide formation. Hoener and DeTurk (10) have shown inferentially that protein is formed by corn plants by way of ammonia to amides to proteins.

Pryanishnikov (17) has concluded that the normal course of protein formation in plants is from ammonia to amides to amino acids to proteins.

When Longfield-Smith (13) exposed half the surface of whole oranges to ammonia atmospheres and protected the other half, he found that the ammonia had practically no effect on the content of acid or the ratio between total soluble solids and acid content. Subsequently it was reported (16) that ammonia is a normal constituent of Valencia oranges to the extent of about 15 p.p.m. in the clarified juice. As ammonia undoubtedly forms salts of organic acids in citrus, and as salts of this kind are assimilated by plant leaves (15), it seems logical to assume assimilation of ammonia by citrus fruits.

Therefore any toxic hazard to be associated with the ingestion of modest residues of increment ammonia in edible plant parts will be minimal. Because Nelson *et al.* (16) reported that a standardized reduce-pressure distillation of their clarified orange juices with 95% ethyl alcohol in the presence of the magnesium oxide afforded highly variable results, however, it was felt necessary to examine the problem of reproducibly determining ammonia residues in considerable detail in order to establish magnitudes of possible persisting residues at time of retail sale.

Companion juice quality (3) and organoleptic tests (4) of ammonia-treated

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fruit were accumulated early in the biological phases of the evaluation of ammonia (17) for the control of the blue-green molds decay of grapefruit, lemons, and oranges. These tests indicated little, if any, deep sorption of ammonia by treated citrus fruits, juice samples from the treated fruits being essentially indistinguishable by standard quality tests (3, 4) from juice samples from the control fruits in every test. Because ammonia treatments may cause wound widening in the peel of injured citrus fruits (19, 20), however, there is sorption of ammonia by the peel, at least after injury. Analytical tests were therefore designed to demonstrate the magnitude of this presumed sorption.

Three types of analytical data have been accumulated: ammonia nitrogen contents of lemons and oranges from nominal ammonia treatment; ammonia nitrogen, total nitrogen, and urea nitrogen contents of lemons from excessive ammonia treatment; and the precise establishment of the magnitudes and rates of ammonia sorption by citrus fruits and by other fruits, vegetables, and eggs with both nominal and excessive ammonia treatment at two temperatures. Detailed study of the fate of ammonia sorbed by the fiberboard that comprises the shipping cartons for citrus fruits was also necessary. The latter study resulted from the conjecture that ammonia sorbed into fiberboard could suddenly be desorbed in sufficient amounts to injure the fruits.

Direct Measurement of Increment Ammonia in Citrus Fruits by Chemical Assay

Ammonia Nitrogen from Nominal Laboratory Treatment. A nominal or recommended commercial treatment of lemons or oranges would consist of not more than 4 grams of ammonia per

Table I. Ammonia Nitrogen in Whole Lemons Treated with Ammonia^a in the Laboratory

Sample	Ammonia Nitrogen, ^{b,c} P.P.M.
0-day control	43 ± 4
3-day control	42 ± 4
Treated	43 ± 4
6-day control	33 ± 5
Treated	38 ± 3
12-day control	48 ± 4
Treated	48 ± 4

^a From ammonia-releasing pellets (8) (Winning-Peplow, Inc., Los Angeles, Calif.).

^b About 500 grams of subsample from the mash of 90 whole fruits was thoroughly disintegrated in a Waring Blendor, 50.00-gram subsamples were placed in 800-ml. Kjeldahl flasks, shaken with 250 ml. of distilled water each, neutralized to methyl red with sodium hydroxide solution, and analyzed as in the standard Kjeldahl procedure (7).

^c 43 p.p.m. = 0.0043%.

carton, released within the carton in not less than 3 and not more than 12 days (19, 20).

Accordingly, lots of 90 lemons each, randomly selected from a commercial run, were treated (17) in standard non-vented cartons with sufficient ammonia-releasing pellets (8) to afford 3.7 grams of available ammonia each. The cartons of treated and control fruits were placed in ventilated storage at 50° F., with control samples near the air inlet and treated samples near the air outlet of the storage room. Separate cartons of both control fruits and treated fruits were analyzed for ammonia nitrogen (7) after storage for 0-, 3-, 6-, and 12-day periods (Table I). These data demonstrate that within the limiting sensitivity of this standard method (7), the treated samples were essentially indistinguishable from the control samples.

A more reproducible general analytical method was therefore devised to demonstrate the presence of ammonia residues in treated fruits. Isolative techniques explored included vacuum distillation with and without alcohol, vacuum and pressure aeration with adjusted pH, normal pressure distillation, ion ex-

Table II. Ammonia Nitrogen from Parts of Lemons and Oranges Treated with Ammonia^a under Commercial Conditions^b

Sample	Ammonia Nitrogen, ^{c,d} P.P.M.
Lemon peel control	22 ± 3
Treated	21 ± 3
Juice control	95 ± 2
Treated	95 ± 2
Orange ^e peel control	16 ± 3
Treated	23 ± 3
Juice control	30 ± 3
Treated	31 ± 2

^a Two wet Winn-Mats Fungicide No. 3 per carton (Winning-Peplow, Inc., Los Angeles, Calif.); each mat contains nine 32-grain ammonia-releasing pellets (8).

^b Telescopic nonvented cartons of lemons and vented cartons of oranges (17) trucked for 2 days under commercial refrigeration (55° to 60° F.), then stored at 50° F. overnight before fruit were processed.

^c Each carton of fruit was randomly separated into four equal subsamples. The juice from three of these was stored at 0° F. in polyethylene bags. The remaining one-fourth carton of fruit was peeled so no pulp adhered, and subsamples of peel were stored at 0° F. in polyethylene bags.

Peel was comminuted with an equal weight of water in a Waring Blendor, and juice was centrifuged. Aliquots of 200 grams in 800-ml. Kjeldahl flasks with a drop of a silicone antifoam agent, 250 ml. of 95% ethyl alcohol, and excess magnesium oxide were diluted with water to 650 ml. Exactly 300 ml. of distillate each was collected in standard sulfuric acid. After evaporation of the alcohol, each solution was back-titrated with standard alkali to a methyl red-methylene blue end point (7).

^d 22 p.p.m. = 0.0022%.

^e Valencia oranges.

change on Permutit (8, 22), and steam distillation. Because of the presence of slowly hydrolyzing nitrogen compounds in citrus juice (10), none of the above methods possessed distinct advantages in cleanly separating the increment ammonia from the persisting dribble of apparent ammonia found in all control samples; full details of these techniques will be reported elsewhere (72). Results from the chosen alcohol-distillation method are shown in Table II for both lemon and Valencia orange peel and juice from fruits exposed to ammonia under commercial conditions.

Again the treated samples were essentially indistinguishable from the control samples, except that the orange peel had acquired 7 ± 3 p.p.m. ($0.0007 \pm 0.0003\%$) increment ammonia under these conditions. This refined chemical assay afforded more reproducible results than the AOAC method (7). Apparently high background values for lemons occur because normal variance of ammonium ion or ammonia progenitors in these fruits is high (72).

Total Nitrogen from Extreme Laboratory Treatment. To determine whether demonstrable amounts of ammonia are sorbed by lemons treated with excessive dosages of ammonia gas, a standard carton of lemons was treated (17) with 11 liters (standard temperature and pressure) of ammonia in 2 minutes. Earlier similar experiments in which the same treatment was given and the carton atmosphere was analyzed every 30 seconds showed that the maximum concentration of ammonia in the free air space within the carton was about 800,000 p.p.m. (77). Treated and control cartons of lemons were stored at room temperature for 3 days; the treated fruit developed the typical (19, 20) severe skin lesions of ammonia burn.

Analytical results for these peel and juice fractions are shown in Table III. The total nitrogen contents of both fractions were significantly higher than control (untreated) values by this excessive dosage. In contrast, subsamples from the whole lemons represented in Table I, afforded total nitrogen values of 1780 ± 20 p.p.m. for the control samples and 1790 ± 20 p.p.m. for the treated samples

Table III. Total Nitrogen from Parts of Lemons Treated with Excessive Amounts of Ammonia under Laboratory Conditions

Sample	Total Nitrogen ^{a,b} P.P.M.
Peel control	2150 ± 50
Treated	2360 ± 20
Juice control	840 ± 10
Treated	900 ± 20

^a Values, for 4 replicates, obtained by method for total nitrogen (7).

^b 2150 p.p.m. = 0.215%.

even after exposure to nominal amounts of ammonia for 12 days at 50° F.

Urea Nitrogen from Nominal Laboratory Treatment. Duplicate subsamples of the 6- and 12-day whole fruit samples of Table I were analyzed for urea nitrogen (Table IV). The 12-day treated sample was indistinguishable from its control sample by this method, but the 6-day sample showed a significant increase in urea nitrogen from the treatment.

Indirect Measurement of Increment Ammonia in Citrus Fruits by Ultraviolet Assay

Residues of increment ammonia from nominal treatment cannot be evaluated by the usual standard methods of chemical assay. However, the total nitrogen method does distinguish between untreated or nominally treated fruits and fruits treated with excessive amounts of ammonia; the latter fruits would be unmarketable because of unsightly ammonia burn (19, 20). Because these standard methods were either too erratic or insufficiently sensitive for the present purpose, a basically different analytical method (7) was adapted for citrus fruits and other commodities for the measurement of nominal amounts of increment ammonia.

The final techniques and equipment used for the present precise evaluation have been described (9). The method is based on the absorption of ultraviolet energy at 204.3 μ by ammonia vapor in air. In principle, an air-ammonia stream flowing at 2 liters per minute was analyzed for ammonia content in a Beckman DK-2 spectrophotometer, then passed into a chamber of fruits held at constant temperature and in a circulating air stream flowing at approximately 350 liters per minute; the air-ammonia mixture surrounding the fruits was drawn out of the system exhaust at 1.5 liters per minute and back through the spectrophotometer for measurement of ammonia depletion. With automatic correction for sorption by the all-glass system, the difference between ingress concentration and egress concentration represented for practical purposes the amount sorbed by the fruit sample. Appropriate calibration of the equipment afforded this value in milligrams of ammonia per unit time; details of construction, operation, and calculations have been presented (9).

Preparation of Samples. All fruits awaiting measurement were stored at 45° F. Fruits to be tested at 50° F. were withdrawn from the cold room, then weighed and measured individually. Measurements were made of the major axis and two minor axes of each fruit to provide the data for determining area and volume in the usual manner (27) for citrus fruits. The fruits were then weighed to 0.1 gram, placed in stainless

steel wire baskets, and lowered into the fruit-sample tubes of the four-unit fruit sorption apparatus (9). Fruits to be tested at room temperature were withdrawn from refrigeration 16 hours in advance of the anticipated determination. Wounded fruits were created by making three random cuts in the peel of each fruit, each cut 1 mm. wide, 1 cm. long, and about 3 mm. deep.

Interpretation and Calculation of Results. With a 17-hour time drive on the spectrophotometer and with operation at a fixed wave length, the completed DK-2 absorption chart consisted of a series of essentially vertical lines, with the vertical span proportional to the differences in the ammonia concentrations (9). Chambers 1 to 4 record across the bottom of the chart; systematic 1-minute air purges are represented by the uppermost row of "points" with every fourth purge of 5-minute duration for keying purposes. Chamber 1 is the empty reference chamber and the points representing it serve as the base line. With constant ammonia concentration, the consecutive points representing air purges will be very nearly parallel to the points recorded from this reference chamber.

The amounts of ammonia sorbed by the commodities are represented by the differences between their respective points and the base line established by the reference chamber on Beckman charts No. 12977; each vertical absorbance unit is equivalent to 0.006 mg. of ammonia per liter of air (7, 9). With a flow rate of 2 liters per minute, the total sorption in terms of milligrams per hour can be readily obtained. The average sorption per hour in milligrams times 1000, divided by the total sample weight in grams, affords the average sorption per unit weight per hour expressed in parts per million.

To illustrate, in a typical run at room temperature with 2195 grams of Valencia oranges and with ammonia at 200 p.p.m., chamber 2 contained unwaxed fruits, and from the chart record the following calculations may be made:

Average Difference from Reference Chamber

		Mg. Sorbed
1st hr.	4.0 abs. units \times 0.006 mg./abs. unit = 0.024 mg./l. \times 120 l./hr.	2.88
2nd hr.	2.5 abs. units \times 0.006 mg./abs. unit = 0.015 mg./l. \times 120 l./hr.	1.80
3rd hr.	2.0 abs. units \times 0.006 mg./abs. unit = 0.012 mg./l. \times 120 l./hr.	1.44
	Total	6.12
	Average	2.04

Therefore, the average ammonia sorbed per hour would appear to be 0.9 p.p.m., if first-hour data are included. As it has been established (9) that first-hour data largely represent dilution of the ingress ammonia by the air in this system, however, the true value of ammonia sorbed per hour is 0.7 p.p.m.

A desorption cycle is calculated in the same manner, but the first hour is consid-

ered to represent dilution of the ammonia atmosphere within the chamber and not true release of ammonia by the fruits. The difference between the sample chamber and the reference chamber therefore represents the net release of ammonia for any given period of time. The instrumental recording of apparently desorbed ammonia seems in part at least to depend upon the presence of free moisture on the commodities or in the system; rates of apparent desorption below about 0.5 p.p.m. per hour may not represent true desorption from the intact tissues of the commodities involved.

Sorption Data for Citrus Fruits. Typical sorption data for ammonia with lemons and oranges are presented in Table V. In contrast to desorption from some commodities other than citrus fruits, the apparent desorption of ammonia by citrus fruits does not present a consistent picture. After correction of the desorption curves for dilution effects (9), even an occasional unwounded sample demonstrated apparently true desorption with sharp decrease in rate. When replicate lots of 1.5 kg. of dry, unwounded mature Eureka lemons, previously exposed to 12,000 p.p.m.-hours of ammonia for 24 hours in an all-glass fumigation chamber were immediately transferred to the air-equilibrated four-unit desorption apparatus, however, chart records showed no detectable desorption (9).

Persisting Residues. The data in Table V permit calculation of residues in the fruits at any time after initial exposure. Consider, for example, the fifth test under Eureka lemons. Five hours' exposure to 475 p.p.m. of ammonia surrounding the fruit affords an ammonia-index value (19, 20) of 2375 p.p.m.-hours. At the end of 5 hours the lemons will have sorbed 16.6 mg. of ammonia. As the sample of lemons in this experiment weighed 1567 grams, the sorbed residue in the fruits at this time amounts to 10 p.p.m. on a total weight basis.

In general, with a given concentration of ammonia surrounding the fruit, the higher the temperature of the fruit the

higher is the rate of sorption of ammonia by the fruit, and, similarly, at a given fruit temperature, this rate increases with increasing concentration of ambient ammonia. Wounded fruits appear to sorb more ammonia than unwounded fruits. Unwaxed fruits appear to sorb at a greater rate than waxed fruits, and immature fruits sorb at a greater rate than mature fruits.

Table IV. Urea Nitrogen from Whole Lemons Treated with Ammonia^a in the Laboratory

Sample	Urea Nitrogen, ^{b,c,d} P.P.M.
6-day control	2 ± 1
Treated	9 ± 3
12-day control	2 ± 1
Treated	3 ± 1

^a From ammonia-releasing pellets (8) (Winning-Peplow, Inc., Los Angeles, Calif.); see Table I for treatment.

^b Replicate 50.00-gram subsamples of ground lemon mixture were placed in 800-ml. Kjeldahl flasks, shaken with 250 ml. of distilled water, and neutralized to methyl red with sodium hydroxide solution; 1 ml. of 20% calcium chloride solution and a few drops of urease enzyme solution were added, and allowed to stand for 1 hour at room temperature. The mixture was treated and analyzed for total ammonia (1).

^c Corrected for free ammonia nitrogen.

^d 2 p.p.m. = 0.0002%.

Indirect Measurement of Increment Ammonia in Other Fruits, Vegetables, and Eggs

Cartons of citrus fruits are often included in mixed shipments of produce, especially in refrigerated trucks. The fiberboard of citrus cartons is permeable to ammonia, and would release ammonia from treated cartons to other commodities present in the mixed shipment. It was therefore of interest to establish the rates of sorption and desorption of ammonia by a variety of other fruits and vegetables that could be in such mixed shipments. Eggs were included because they are frequently shipped this way.

Tests were performed in the apparatus and in the manner described. All samples were mature items purchased from a local wholesale house. From the typical sorption data shown in Table VI it is clear that ammonia is sorbed by a large variety of these commodities; with some of them sorption from a nominal atmosphere of ammonia is sufficiently rapid and extensive to produce ammonia injury. Sorption is particularly high when the items are bruised, wounded, or cracked; this presumes sorption by way of exposed moist tissues. Evidence for desorption is strong for some of these commodities, however, and thus implies decreased persisting residues of ammonia after aeration. Among these commodities, only the exposed strawberries had off-flavor, and only the strawberries were visibly damaged by the treatment with ammonia. This point is of interest because pronounced injury to a variety of fruits and nuts (2, 78) and to onions (78) from very high concentrations of ammonia has been reported.

"Buffer" Action of Fiberboard

The possibility that the fiberboard comprising the storage and shipping cartons acts as an ammonia reservoir was of

Table V. Typical Sorption Data for Ammonia with Mature Citrus Fruits^a

T., ° C.	Ambient NH ₃ , P.P.M.	Rate Sorbed from Air Stream, P.P.M. NH ₃ /Hr.			Apparent Rate Desorbed, P.P.M. NH ₃ /Hr.
		Waxed ^b	Unwaxed	Wounded ^c	
Eureka Lemons					
12	220	1.2, 1.1	1.7	...	<0.5
	535	6.3 ^d	2.4 ^e	3.6	...
	725	8.1 ^d	3.0 ^e	4.3	...
	27	242	1.6	1.6	...
27	475	2.1	...	3.2, 3.1	<0.5
	525	3.4, 3.4	3.3	...	<0.5
	725	4.3 ^d	4.2
	27	242	1.6	1.6	...
Navel Oranges					
12	175	0.2	0.4, 0.4
	460	1.0	1.0, 1.0
23	185	1.1	1.0
	630	1.5	2.4, 2.0
10	175	0.2 ^f	0.4, 0.4 ^f	...	<0.5
	460	1.0	1.0, 1.0	...	<0.5
	775	1.0, 1.0 ^g	1.0 ^g	...	<0.5
Valencia Oranges					
10	340	1.0	1.2	1.6	<0.5
	540	0.8	1.1	2.1	<0.5
27	200	0.7	0.9	1.1	<0.5
	510	1.6	1.8	2.5	<0.5
Marsh Grapefruit					
14	260	0.9, 0.7	...	3.4	0.1, 0.3
	490	0.8, 0.6	...	3.8	0.4, 0.3
27	220	0.7, 1.0	...	4.1	0.5, 0.4

^a Approximately 2 kg. per test; data include first hour of sorption (see text).

^b Waxed in commercial packing house.

^c Each fruit had three random cuts in the peel, 1 mm. wide, 1 cm. long, about 3 mm. deep.

^d Silvers.

^e Light greens.

^f Same lot of fruits but cured for 2 weeks under commercial conditions.

^g Freshly picked and washed fruits.

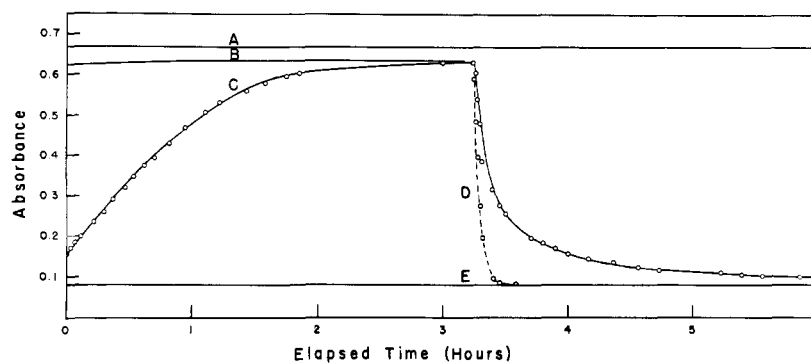


Figure 1. Typical fiberboard sorption-desorption curve

concern. For example, if the carton components sorb ammonia with retention at low temperatures but with release at high temperatures, it is conceivable that removal of cartons from refrigerated storage could release enough ammonia to burn the fruits (19, 20). It was also of interest to establish the "buffer" capacity of fiberboard with respect to ammonia sorption-desorption, because a high capacity would simplify the practicable requirements for maintenance of the ammonia atmosphere surrounding the fruits for mold control (19).

Typical samples of fiberboard representing the components of citrus cartons were therefore obtained from major manufacturers of these cartons. Top and

bottom portions of the two-piece commonly used telescoping fiberboard cartons were tested separately. Each carton part—i.e., top or bottom—was weighed and then cut at right angles to the corrugations into strips 8 inches long by 3/4 inch wide. Bundles consisting of 10 such strips were weighed and stored at room temperature for 24 hours in a humidifying chamber containing a saturated solution of ammonium sulfate to provide a relative humidity of 80%. The component parts of various fiberboards, as well as standard cellulose extraction timbles to evaluate sorption by pure cellulose, were conditioned in a like manner. All samples were evaluated as previously described (9).

Table VI. Sorption of Ammonia by Fruits, Vegetables, and Eggs

Item	T, ° C.	Sample, Weight Kg.	Ammonia		
			Ambient p.p.m.	P.P.M./Hr.	
				Sorbed	Desorbed
Apples, green cooking	12	1.5, 1.6, 1.4	135 430	2.2, 3.1, 2.1 3.1, 5.9, 4.9	... 1.5, 0.8, 2.3
Apricots, Dumont Royal	12	1.7, 1.6, 1.8	110 455	3.7, 3.7, 3.5 10.5, 10.8, 10.9	... 0.5, 0.4, 0.3
Asparagus, green	12	1.1, 1.1	100 535	9.8, 9.6 34.3, 33.9	... 1.1, 1.3
Avocados, Fuerte	12	1.3, 1.3, 1.3	125 510	2.2, 3.5, 3.1 4.2, 8.3, 6.1	... 1.1, 0.7, 1.5
Bananas, Central America	27	1.4, 1.4	95 470	2.8, 2.1 7.0, 7.3	... 1.4, 1.8
Beans, Wade Green	12	0.9, 0.9, 0.9	510	27, 31, 33 ^a	0.4, 0.6, 0.8 ^a
Carrots, Danvers Half-Long	12	1.6, 1.7	450	17.5, 17.6	1.0, 0.8
Celery, Utah unbleached	12	0.8, 1.1, 1.1	95 360	7.5, 8.0 11.4, 18.1	... 1.4, 0.7
Cherries, Tartarian	12	1.2, 0.6	440	21.2, 20.5	0.5, 0.7
Corn, yellow sweet	12	1.5, 1.8, 1.5	125 500	9.0, 9.1, 6.0 ^b 24.4, 24.6, 16.7 ^b	... 1.5, 0.9, 0.9 ^b
Cucumbers, extra fancy	12	2.0, 2.0, 1.4	105 515	2.4, 2.8, 4.0 ^c 5.0, 6.0, 8.4 ^c	... 0.5, 0.8, 1.2 ^c
Eggs, White Grade A	12	1.2, 1.2, 1.2	125 500	1.6, 1.8, 4.0 ^d 2.6, 3.2, 8.0 ^d	... 1.4, 1.5, 2.6 ^d
Peaches, Red Haven	12	1.4, 1.3	470	6.8, 7.8	0.5, 0.6
Peppers, Green Bell	12	1.0, 1.0	500	5.9, 6.9	1.3, 1.6
Potatoes, Irish	12	1.8, 2.0, 2.0	125 510	2.0, 1.7, 2.7 4.5, 5.4, 5.0	... 0.6, 0.6, 0.8
Squash, Yellow Crookneck	12	1.3, 1.3, 1.5	115 480	5.6, 5.7, 6.0 11.0, 12.1, 12.8	... 1.2, 1.3, 1.1
Strawberries ^e , Univ. Variety	10	0.7, 0.6, 0.7 0.7, 0.8, 0.7	105 520	15.1, 17.6, 15.8 44.5, 43.5, 37.6	0.5, 0.6, 0.7 0.7, 0.8
Tomatoes, Vine-ripened	10 12	1.1, 1.1, 1.1 1.8, 1.2, 1.4	520 470	1.6, 2.3, 5.5 ^f 1.7, 6.7 ^f	1.0, 0.7, 1.9 ^f 0.5, 1.3 ^f
Turnips, Purple-top	12	1.1, 1.2, 1.0	100 505	7.8, 7.3, 9.0 20.4, 20.5, 23.0	... 1.5, 1.3, 2.2
Turnip greens, Purple-top	12	0.2, 0.2, 0.2	105 495	52.6, 47.0, 48.8 165, 153, 158	... 9.0, 8.1, 7.7

^a Snapped; other replicates not snapped.
^b Shucked; other replicates not shucked.
^c Wounded and bruised; other replicates not deliberately wounded and bruised.
^d Cracked lightly; other replicates not cracked.
^e Threaded and suspended in sample chambers.
^f Bruised and split; other replicates not deliberately bruised and split.

Calculations. The determination of ammonia sorption and desorption is illustrated by the typical fiberboard sorption-desorption curve reproduced in Figure 1. The base reference line, *E*, is the line indicating instrument blank level as determined at the start of the run. The upper reference line, *A*, is placed at 0.675 absorbance unit above the instrument blank line; at this point an absorbance of 0.675 above the instrument blank is equivalent to 0.4 mg. of ammonia per liter of air-ammonia mixture. At a flow rate of 2 liters per minute for 200 minutes area *W* of the chart between the base reference line and upper reference line from 0 to 200 minutes is equivalent to 160 mg. of ammonia. A tracing of the entire plot is made on onion skin paper. The area bounded by the

absorbance axis, *A*, *D*, and the time axis up to 200 minutes, area *X*, is cut out and weighed. Next, area *Y*, bounded by the absorbance axis, *B*, and curve *C*, which consists of the portion of the chart between the plot of ingress ammonia and the sorption curve, is cut out and weighed. Then,

$$\frac{[\text{Wt. of area } Y \text{ (gram)}] \times (160 - \text{i.e., mg. NH}_3 \text{ equivalent to area } W)}{[\text{wt. of area } X \text{ (gram)}]} =$$

NH₃ equivalent to area *Y* (mg.), which is mg. NH₃ sorption by the sample used.

$$\frac{\text{Wt. of carton part (gram), top or bottom}}{\text{wt. of sample used (grams)}} =$$

factor to carton part

$$(\text{Factor to carton part}) \times (\text{mg. NH}_3$$

sorption by sample used) = total sorption of mg. NH₃ per carton part

$$\frac{\text{Total sorption of NH}_3 \text{ per carton part (mg.)} \times 1000}{\text{wt. carton part (grams)}} =$$

NH₃ sorption in mg. per kg. carton part

The desorption calculations are made in a similar manner. The relationship between the weight of area *W* and of the area representing the amount of ammonia desorbed is established. The desorption area, *Z*, is taken as the area between the instrument blank level and the desorption curve. The weight of area *Z* is modified by a constant dilution factor which is plotted on the chart (*D*) and cut out before area *Z* is cut out and weighed.

This dilution factor is obtained when the apparatus is in equilibrium by shutting off the ammonia from the incoming air-ammonia mixture. The descending ammonia concentration is then plotted against time as curve *D*.

Curves similar to that shown in Figure 1 were drawn for all triplicated samples of fiberboard and of fiberboard components as tested at 77°, 54°, and 32° to 39° F.

The moisture correlation coefficient was also established; this is a numerical value designed to show if a possible correlation exists between moisture content as shown by loss on drying and ammonia sorption:

$$\frac{\text{NH}_3 \text{ sorption (in mg. per kg. carton part)}}{\% \text{ moisture loss}} = \text{moisture correlation coefficient}$$

Results. In Table VII are collated the summary data of these tests on the ammonia sorption and retention properties of fiberboard and fiberboard components. Some of these test materials represent samples of fiberboard from commercial runs; others represent components used in the manufacture of various types of fiberboard for citrus cartons, selected to include permissible ranges in quality and composition.

In Table VIII are collated both sorption and desorption data for commercial telescoping carton parts exposed to ammonia at three temperatures. These data demonstrate a trend toward correlation of ammonia sorption-desorption with temperature, although some of the products appear to be temperature-independent in this regard.

Pure cellulose sorbed 173 mg. of ammonia per kg. in 200 minutes at 77° F. and desorbed 128 mg. of ammonia per kg. in 200 minutes at 77° F., the moisture correlation coefficient for this cellulose from extraction thimbles was 20.9 (Table VII).

While it seems logical that ammonia sorption by any one paper stock will vary with the moisture content, and hence with the relative humidity of the ambient atmosphere, study of Table VII shows that moisture contents of different stocks in equilibrium with air of constant hu-

Table VII. Samples of Fiberboard

Mfg. Code	NH ₃ Sorption, Mg./Kg.	Moisture		Mfg. Code	NH ₃ Sorption, Mg./Kg.	Moisture	
		% ^a	Correlation coefficient			% ^a	Correlation coefficient
A. Gaylord Container Corp.				45#	896	12.11	74
33#	786	10.28	76	69#	670	9.28	72
45#	610	9.83	62	76#	1630	10.88	150
47#	552	8.93	62	E. Longview Fibre Co.			
69#	567	10.64	53	Silverado	1027	11.38	90
B. Gaylord Kraft					1097 ^b	9.93	110
2-16-56	764	10.57	72	1-47	1117	20.14	55
1-11-56	999	10.75	93		1623	12.22	133 ^c
1-29-56(39)	1001	11.40	88	1-72	1164	10.09	115
1-30-56(39)	1191	10.43	114		1140 ^b	10.44	109
2-14-56(39)	927	7.14	130	4-32	1212	11.11	109
1-31-56(60)	895	11.38	79		1128	11.28	95
1-14-56(60)	783	11.44	68	6-45	955	9.98	96
1-30-56(60)	1140	11.46	99	6-47	873	25.58	34 ^c
1-21-56(60)	756	11.35	67	F. Menasha Container Corp.			
1-31-56(60)	997	10.81	92	33#	484	9.90	49
1-22-56(60)	600	10.80	56	47# O	835 ^b	9.49	88
C. Kieckhefer Container Co.				47# I	858 ^b	9.47	91
45#	1023	11.79	87	69#	849 ^b	10.41	82
69#	969	11.47	84	G. Weyerhaeuser Timber Co.			
Corr. Med.	972	12.08	80	26#	629	9.91	63
45# Silverado	1840 ^b	10.62	173	33#	656	10.00	66
D. Kieckhefer, N. C., Pulp				42# PV	880 ^b	10.30	85
Silverado	1793 ^b	19.19	93	42# WP	1435	10.31	139
26#	619	10.76	58	42# WF	819	10.71	76
33#	1578 ^b	11.14	142	42# WV	1361	10.70	127
33#HSC	1122	10.45	107	69# HS	664	11.08	60
A. 33 #/M sq. ft. semichemical	E. Liner board						
45 #/M sq. ft. Silverado	Natural plain cylinder liner grade 1-47						
47 #/M sq. ft. Fourdrinier kraft	Natural plain cylinder liner grade 1-72						
69 #/M sq. ft. Fourdrinier kraft	Natural plain cylinder corrugating medium grade 4-32						
B. Grade Silverado mill dates 2-16-56, 1-11-56	Secondary headbox lined board white 60-1128 grade 6-45						
Grade 39 mill dates 1-29-56, 1-30-56, 2-14-56	Natural plain Fourdrinier liner grade 6-47						
Grade 60 mill dates 1-31-56, 1-14-56, 1-30-56, 1-21-56, 1-31-56, 1-22-56	F. 33-# corrugating medium						
C. 45-# liner	47-# Silverado outside liner						
69-# liner	47-# kraft inside liner						
Corrugating medium	69-# outside liner						
45-# Silverado	G. 26-# Semi-Kern corrugating medium reel 6702 Concora 66 Porosity 22.5						
D. Liner board	33-# Semi-Kern corrugating medium reel 6676 Concora 79.5 Porosity 26.5						
26-# kraft new sol. corrugation	42-# ply veneer						
33-# kraft	42-# Weyer Pak						
33-# high size corrugation	42-# water finish						
45-# kraft	42-# Weyer Vee						
69-# kraft	69-# high size						
76-# Ply Pak							

^a Dried at 100° C. for 5 hours. ^b Equilibrium not completely established. ^c Possible error in moisture loss.

Table VIII. Typical Sorption and Desorption of Ammonia by Carton Parts at Different Temperatures

Mfg. and Carton Part	(Mg./kg.)					
	32° F. ^a		54° F. ^b		77° F. ^c	
	Sorption	Desorption	Sorption	Desorption	Sorption	Desorption
Longview 22						
Top	1142	729	956	596
Bottom	1142	620	1091, 1174 ^d	...
International Paper 43						
Top	1671	618	1372	518	1225	...
Bottom	1517	607	1392	503	1545	...
General Container 3A						
Top	814	466	658	...
Bottom	851	510	649	479
Menasha 2V						
Top	1180	608	1200	610	920	516
Bottom	1393	682	1046	623	778	528

^a Single run. ^b Av. of two replicates. ^c Av. of three replicates. ^d At 100° F., single run.

midity are not even an approximate indicator of the quantity of ammonia that the stock will sorb.

Persisting Residues. Detailed evaluations of the rates of sorption and desorption of ammonia by the fiberboard cartons at constant ammonia concentration in air of 80% relative humidity were made with over 100 types of fiberboard and fiberboard components. These evaluations demonstrate the validity of the initial assumption that fiberboard acts as a "buffer" in the present usage, in that it helps resist sudden changes in both the ammonia and moisture contents of the atmosphere within cartons of treated citrus fruits. Within the temperature range utilized, sorption of ammonia was not completely reversible, for desorption afforded the return of from 1/2 to 3/4 of the initially sorbed ammonia. Rates of sorption and total amounts sorbed were not directly related to the moisture contents of the fiberboard samples, thus implicating H-bonding as a factor in this type of sorption.

Conclusions

Concentrations of ammonia multiplied by time sufficient to cause chemically determinable increments in nitrogen residue in lemons usually result in severe fruit burns (19, 20); this self-limiting feature of the use of ammonia has been observed (17) in semicommercial tests.

Freshly wounded fruits and vegetables appear to sorb appreciably more ammonia than unwounded items. With citrus fruits, however, ammonia sorbed into a fresh wound causes the wound to widen markedly and to become dry and brown in appearance. Thus, this treatment may permit the locating and discarding of perhaps not otherwise detectably wounded, weak fruit at the retail level and possibly at the jobber level. Mixed refrigerated shipments of ammonia-treated citrus fruits with other perishable commodities might damage some of them, particularly strawberries.

Within the effective parts per million-hour range (19, 20) and below the concentration of ammonia which results in tissue damage, the sorption of ammonia by citrus fruits may be described as follows:

Rate of sorption is nearly uniform for any concentration of ammonia, and is constant for a given concentration of ammonia for at least 12 hours of treatment. An apparently high initial rate of sorption is actually the dilution of the ingress ammonia by the mass of air within the sample chamber.

Mature fruits, unwounded fruits, and waxed fruits sorb ammonia at lower rates than immature fruits, wounded fruits, and unwaxed fruits.

The lower the temperature of the fruits during treatment with ammonia, the lower the rate of sorption of ammonia by the fruits.

The desorption of ammonia by citrus fruits presents a confused picture. Much of this apparent desorption is probably simple dilution, by fresh air, of the ammonia-air mass surrounding the fruits inside the experimental chambers. Not all the instances of apparent desorption can be accounted for by this simple explanation, however, for some moist or wounded fruits and vegetables unquestionably desorb measurable ammonia after treatment.

The fiberboard composing citrus shipping cartons also sorbs ammonia during the treatment, then desorbs the major part of the contained ammonia into an ammonia-free atmosphere. As with many of the fruits and vegetables tested, the desorption process with fiberboard is essentially complete within a few hours after initiation. The many types of fiberboard and fiberboard components examined in detail exhibited very similar sorption characteristics. Ammonia sorption by fiberboard is not directly related to moisture content.

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Literature Cited

- (1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 7th ed., pp. 370-1, 1955.
- (2) Dewey, D. H., *Ice & Refrig.* **123**, 19 (1952).
- (3) Eaks, I. L., personal communication.
- (4) Ewart, W. H., personal communication.
- (5) *Federal Register*, **21** (144), 5619 (1956).
- (6) Folin, O., Bell, R. D., *J. Biol. Chem.* **29**, 329 (1917).
- (7) Gunther, F. A., Barkley, J. H., Kolbezen, M. J., Blinn, R. C., Staggs, E. A., *Anal. Chem.* **28**, 1985 (1956).
- (8) Gunther, F. A., Blinn, R. C., Barkley, J. H., Kolbezen, M. J., Staggs, E. A., *J. Agr. Food Chem.* **7**, 489 (1959).
- (9) Gunther, F. A., Blinn, R. C., Kolbezen, M. J., Wilson, C. W., Conkin, R. A., *Anal. Chem.* **30**, 1089 (1958).
- (10) Hoener, I. R., DeTurk, E. E., *J. Am. Soc. Agron.* **30**, 232 (1938).
- (11) Klotz, L. J., unpublished data for Project No. 808, University of California, Riverside, Calif.

- (12) Kolbezen, M. J., manuscript in preparation.
- (13) Longfield-Smith, L., *Florida State Chemist*, Ann. Report 1932, 22.
- (14) McFarland, F. J., letter to A. M. Boyce, Jan. 9, 1957, relative to F. D. A. Petition No. 86 and supplement.
- (15) Mothes, K., *Planta (Abt. E. Z. wiss. Biol.)* **19**, 117 (1933).
- (16) Nelson, E. K., Mottern, H. H., Eddy, C. W., *Fruit Products J.* **12**, 231 (1933).
- (17) Pryanishnikov, D. N., *Bull. Acad. Sci. U.R.S.S., Ser. Biol.* **1945**, 147.
- (18) Ramsey, G. B., Butler, L. F., *J. Agr. Research* **37**, 339 (1928).
- (19) Roistacher, C. N., Klotz, L. J., Eaks, I. L., *Calif. Agr.* **11** (10), 11 (1957).
- (20) Roistacher, C. N., Klotz, L. J., Kolbezen, M. J., Staggs, E. A., *Phytopathology* **47**, 533 (1957).
- (21) Turrell, F. M., "Tables of Surfaces and Volumes of Spheres and of Prolate and Oblate Spheroids, and Spheroidal Coefficients," Univ. of Calif. Press, Berkeley and Los Angeles, 1946.
- (22) Whitehorn, J. C., *J. Biol. Chem.* **56**, 751 (1923).

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INSECTICIDE RESIDUES

The Endrin Content of Eggs and Body Tissue of Poultry Receiving Endrin in Their Daily Diet

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The tissues and eggs of chickens ingesting small amounts of endrin daily were examined for endrin residues using a specific spectrophotometric method of analysis. Results were confirmed with a nonspecific bioassay method sensitive to 0.02 p.p.m. of endrin. At the lowest level of intake, 0.1 p.p.m., endrin could not be detected in the eggs, but was present in the body fat. Both tissues were contaminated with endrin at higher levels of intake.

THE fate of insecticides in the animal body is a subject of considerable importance in modern pest control. Because many contemporary pesticides possess residual properties which make them effective for weeks or months, they can conceivably be distributed rather widely from their original site of application. A material used on forage or grain crops, for example, may be present as minute residues on the harvested crop and eventually become deposited in the tissues of the animal eating these feed-stuffs. The results of a study of the distribution of endrin (1,2,3,4,10,10-

hexachloro - 6,7 - epoxy - 1,4,4a,5,6,7,8,8a - octahydro - 1,4 - endo - endo - 6,8 - dimethanonaphthalene) in poultry tissue are described in this paper.

The fate of insecticides applied to or consumed by poultry has been investigated by several workers. Hoskins (9) and Furman and Bankowski (5) have described the absorption of benzene hexachloride by chickens following treatment of their roosts with various formulations of this compound. Draper and coworkers (3) have found that after 2 years of DDT intake the DDT level in poultry fat is approximately 10 times

that of the diet. When Sherman and Rosenberg (13) fed isodrin at 3.5 mg. per kg. of diet and endrin at 4.3 mg. per kg., they found 0.6 p.p.m. of isodrin and 6.7 p.p.m. of endrin in the fat 24 hours later. Fairchild and Dahm (4) reported that when chickens were given a single dust application of lindane or dieldrin, dieldrin persisted in the body fat for as much as 12 weeks with residue levels higher than those found at one week. In the case of lindane, residues of 11 p.p.m. were found one week after dusting with a steady decrease to about 0.3 p.p.m. at 8 weeks. Marsden