nitrile extract at the rate of about 5 ml. per minute, with the aid of slight suction, through a column of acid-washed alumina prepared as described under "Apparatus." The amount of coloring matter varies from sample to sample and it may be that one column can be used for several samples or that more than one column will be required for one sample. Do not allow the level of the acetonitrile to fall below the level of the alumina. After the extract has passed through, wash the column with 50 ml. of acetonitrile. Concentrate the combined acetonitrile filtrate to about 25 ml. over a steam bath with the aid of a jet of air.

Transfer the acetonitrile to a 500-ml. separatory funnel with the aid of two 25-ml. portions of carbon tetrachloride. Add 75 ml. of aqueous 9% sodium sulfate solution, 5 ml. of 12N hydrochloric acid, and 75 ml. of water to the funnel and shake vigorously for 1 minute. Filter the carbon tetrachloride layer through a fluted filter paper into a dry, 250-ml. separatory funnel, but do not allow any of the aqueous layer to run onto the paper and do not wash the paper. Add 50 ml. of carbon tetrachloride to the 500-ml. separatory funnel containing the aqueous layer and shake vigorously for 1 minute. Filter the carbon tetrachloride layer into the 250-ml. separatory funnel and discard the aqueous layer. Add 1 ml. of carbon disulfide solution and 25 ml. of ethyl alcohol to the funnel and mix by swirling. Add 1 ml. of 6N sodium hydroxide and shake for exactly 1 minute. Continue the procedure as for the preparation of the calibration curve beginning with, "Immediately,

add 75 ml. of 9% aqueous sodium sulfate solution. . . . etc." Extract the aqueous, acidified layer containing the dimethyldithiophosphoric acid four or more times with 25-ml. portions of carbon tetrachloride until the carbon tetrachloride is proved colorless by measuring the absorbance at 418 mμ. These extractions remove the interfering colored substances originally present in the sample and the xanthates formed by the addition of carbon disulfide in a previous step. Determine from the calibration curve the amount of malathion corresponding to the observed absorbance.

Discussion

Malathion residues will probably be concentrated in the oil; therefore, quantitative extraction of the oil from cottonseed seemed desirable. n-Hexane was chosen as the solvent, because the oil and the malathion are both readily soluble in it. Experiments indicated that Soxhlet extraction for 4 hours with n-hexane at the rate of about 2.7 liters per hour resulted in essentially complete removal of malathion and oil from 200-gram samples of pulverized seed. To provide a safety factor, a 6-hour extraction has been recommended.

Acetonitrile is used to extract the malathion from the hexane–cottonseed oil solution, because malathion distributes very favorably between the two solvents. Four 50-ml. portions of acetonitrile are sufficient to remove the malathion from 350 ml. of hexane-oil solution.

Acetonitrile extracts from the hexane-

oil solution an acidic yellow substance (probably gossypol) which causes high control values on samples containing no malathion and also produces no or low recovery on those containing added amounts of malathion. Acid-washed alumina, used previously for a similar purpose (7), proved an excellent adsorbent for these interfering colored materials, while malathion was not adsorbed. A straw-colored pigment is not removed by the alumina, but this does not interfere, because it is removed at a later step.

The calibration curve between 25 and 150 γ is essentially a straight line with a slope of 3.2 (absorbance per milligram of malathion). Below 25 γ, it gradually changes slope and passes through the origin.

Control tests should be run whenever possible on samples known to contain no malathion and suitable corrections should be applied on results obtained for treated samples. Based upon the data presented, the method described appears satisfactory for determining malathion residues in cottonseed in concentrations down to 0.1 p.p.m. and in field-treated samples.

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FUNGICIDES FOR PACKAGED FOODS

Generation of Ammonia for In-Package Fumigation of Foodstuffs

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Ammonia gas is fungicidal to some common mold organisms, but bulk fumigation with this gas may not afford the long-term protection sometimes required under commercial conditions. In-package generators of ammonia have been developed to maintain critical gas concentrations surrounding a fresh commodity over controlled periods, activated by the highly humid environment within a package of actively respiring plant parts. Two types of practical generators are discussed, with applications to citrus fruit handling. The first involves simple hydrolysis of diammonium succinate; the other utilizes the moisture-initiated reaction between dry ammonium sulfate and dry soda ash.

A MMONIA GAS is fungicidal to some common mold organisms, including the blue-green molds, *Penicillium digitatum* Saccardo and *P. italicum* Wehmer, which commonly attack citrus fruits after harvest, during shipment, and in storage (21). Decay by these organisms involves germination and vegetative growth

of their air-borne spores which have lodged in fresh wounds in the rind of fruits. Methods of mitigating this decay (14) have included dipping fruits into fungicidal solutions or suspensions, but existing fungicidal materials afford little residual protection against reinfection by air-borne spores in fresh wounds

caused by handling after dipping. However, fumigation with a suitable material after fruits have been packaged protects against this reinfection; biphenyl is currently being used for this purpose.

Citrus fruits packed in fiberboard cartons may be protected with gaseous ammonia (21, 22), which according to Klotz
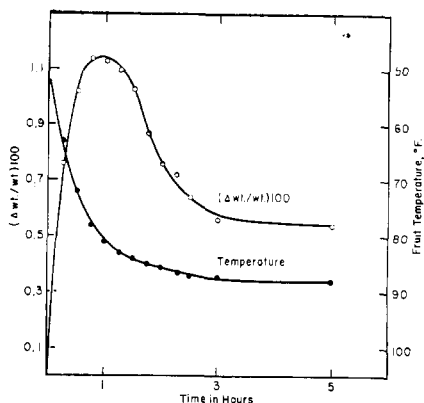
Figure 1. Time-temperature and time-moisture relationships of oranges suddenly transferred from 50° F. and 95% relative humidity to 88° F. and 92% relative humidity

Experimental values are averages of 5 replicates with oranges 6 cm. in diameter and weighing 106 grams each. Lemons behaved similarly

Table I. Comparative Temperatures and Relative Humidities within Standard Cartons of Citrus Fruits[a]

| | Room | | Inside Carton | | | |
| | | | Refrigerated | | Not Refrigerated | |
	Temp., ° F.	% R.H.	Temp., ° F.	% R. H.	Temp., ° F.	% R.H.
Lemons[b]	70–80	25–50	47–51	97–99	67–75	97–99
Oranges[c]	69–80	11–31	47–54	84–98	68–74	50–100[d]

[a] Range values from continuous cross-calibrated hygrothermograph records over 12-day period. [b] In nonvented cartons. [c] In vented cartons. [d] Much diurnal fluctuation, with gradually decreasing relative humidity inside cartons.

(12) is fungicidal to these mold organisms. Bulk fumigation with ammonia may not always afford the long-term protection sometimes required (22) under commercial conditions, however, and in-package generation of ammonia is sometimes advantageous, to cope more adequately with highly variable commercial conditions. As a practical fumigant for foodstuffs ammonia has many desirable features. In addition, the generally recognized low mammalian toxicity of ammonia and many of its salts, which could be present as potential residues in treated edible commodities, ensures favorable consideration of this fumigant as a pesticide under Public Law 518. In its applications to control the blue-green molds decay of citrus fruits, for example, under this law ammonia has been declared exempt from the requirement of a tolerance.

The maintenance of critical concentrations of ammonia at the required times and for the required periods within fiberboard cartons of citrus fruits poses a difficult problem, for the environment within such a carton represents a dynamic system (5). The present in-package generators of ammonia will probably find applications wherever fungi susceptible to ammonia are concerned. They consist of compressed pellets capable of being activated by the highly humid environment within a package of actively respiring citrus fruits. Depending upon times and rates of ammonia release desired, however, other forms may be useful for other conditions with, for example, other fruits in different types of containers.

This report is concerned with the evolution of the present forms of these ammonia generators and their practical laboratory evaluations, with citrus as an example of usage.

Evolution of Present Ammonia Generators

For practical fungus control with packaged fresh fruits and vegetables, the ideal ammonia generator would be activated only by the in-package environment; otherwise the shelf lives of the generators would be short and their manufacture difficult. A compound or mixture of compounds capable of evolving ammonia upon absorption of moisture in the highly humid environment within a package of actively respiring plant parts might protect them from decay caused by organisms susceptible to ammonia. This evolution of ammonia could result either from simple hydrolysis or from other chemical reaction induced by the abundant moisture inside a packed carton of fruits. For example, the relative humidity inside standard nonvented cartons of lemons and standard vented cartons of oranges under several temperature conditions is considerably above that of the external environment (Table I). A typical rate at which free moisture collects on the surfaces of chilled (50° F.) citrus fruits suddenly transferred to a warm (88° F.) and humid (92% relative humidity) storage environment is shown in Figure 1. Under the former conditions there is only about 0.4 gram of water in the atmosphere within a standard packed carton of 200-size fruits, but the respiring fruits will maintain and increase (Table I) this reservoir of water; under the latter conditions, there are approximately 115 grams of condensed water on the fruit surfaces within the carton within 1 hour.

Three types of ammonia generators activated by moisture were investigated.

Enzymatic. This type involved incorporating a dry enzyme preparation with a potential source of ammonia— e.g., urease with crystalline urea. As ammonia could not be obtained in useful quantities from this type of reaction, other types of generators seemed more practicable.

Hydrolytic. Simple humidity-induced and alkaline hydrolyses of 15 amides, including mono-, di-, and tri-basic types, were explored; neither type of hydrolysis under the conditions of the present problem afforded encouraging results. The simple hydrolysis of ammonium salts, however, did provide encouraging results. In Table II are

listed the ammonium salts of the weak acids prepared and evaluated as generators of this type. Ionization constants are included because, from a theoretical standpoint, the salts of the weaker acids would hydrolyze to a greater extent and thus yield greater vapor tensions of ammonia during hydrolysis. Also, salts derived from weak acids and ammonium hydroxide generally yield ammonia vapors due to simple dissociation into the acid and ammonia (7). The concentrations of ammonia in the air-vapor mixtures over these dry salts are shown in Table III; true concentrations of ammonia in the vapor phases in equilibrium with the pure salts are bracketed by the values given in the last two columns. The undissociated values may incorporate contributions by vapors of the parent acid, whereas the dissociated values are probably overcorrected because the concentration of the acid moiety would presumably be lower over the salt than over the free acid.

Table III shows that a wide choice of ammonia concentrations in equilibrium with such salts is available, ranging from less than 0.1 mg. per liter over diammonium malonate to 3.3 over diammonium pimelate. Practicable control of the common blue-green molds decay of citrus fruits with ammonia, however, requires long-term exposure to ammonia surrounding the fruits within the range 300 to 6000 p.p.m.-hours as determined by a Nessler test (13, 22). Evaluative biological screening of all these salts demonstrated that diammonium adipate, pimelate, sebacate, suberate, and succinate afforded promising control of these two molds (13). Organoleptic, economic, and toxicological considerations indicated that diammonium succinate should be most intensively evaluated.

Diammonium succinate is generally considered to be nontoxic; it contains 22.4% ammonia nitrogen, and is easily made from materials available in quantity. It is deliquescent, but in the presence of moisture alone it releases approximately half its available ammonia, presumably forming the moisture-stable monoammonium acid salt at room temperatures. In the presence of moisture plus mild alkali, however, it releases all its ammonia.

Metathetical. For economical rea-

sons the metathetical-type generators ammonium biborate, ammonium chloride, ammonium sulfate, diammonium adipate, and diammonium succinate were evaluated most intensively. Ammonium nitrate was not used because it is both hygroscopic and explosive; ammonium sulfide was eliminated because of its stench. Intimate admixtures of these salts with each of the weakly basic substances, calcium oxide, magnesium oxide, sodium carbonate (soda ash), or sodium sesquicarbonate (20), resulted in moisture-sensitive ammonia generators (7). The use of disodium adipate or succinate, monosodium adipate or succinate, or sodium ammonium adipate or succinate in admixture with the corresponding diammonium salts afforded very rapid release of available ammonia in the presence of moisture, but these alkaline salts are synthesized with difficulty.

The use of essentially anhydrous ammonia sources and alkaline materials assured long storage life as well as essential freedom from reaction during formulation. Consistent control of rates of generation of ammonia in the presence of moisture could most easily be achieved by pelletizing, the amount of ammonia depending upon the number of uniform pellets.

Miscellaneous. Saturated solutions of ammonia in isopropyl alcohol as well as various concentrations of copper ammonia complexes in aqueous solution were also tested in several formulations. Both were considered impractical, in that the former was difficult to store and unpleasant to handle, whereas the latter could leave explosive residues upon drying.

Preliminary Formulations. Small scale tests confirmed by extensive large scale and commercial tests (13) demonstrated the feasibility of the in-package generation of ammonia for the present purpose. Loose powders sprinkled over the fruits are not completely satisfactory because of possibilities of unsightly deposits, uncontrollable rates of ammonia release, localized high concentrations of ammonia resulting in fruit burn, and simple chemical burn from direct contact with the fruit rind (7, 13). Other methods tested included saturated solutions poured onto paper toweling, powdered material enclosed in paper toweling packets, powdered material suspended in citrus slab-wax then brushed onto the fruit, and pellets (7).

Ammonia-releasing chemicals mechanically mixed with diluents and extenders with or without alkaline reactants frequently afforded undesirable and uncontrollable flash releases of ammonia in test cartons of fruits, especially at room temperatures. On the other hand, pelletizing these mixtures or otherwise granulating them with pressure resulted in some degree of control of hydrolysis rates through degree of compression, sizes of

Table II. Ammonium Salts Prepared from Organic Acids

Acid Used	% NH₃ Theory	% NH₃ Found^a	Solvent Used in Preparation,^b Ml.	Acid Ionization Constant^c	Lit. Synthesis
Aliphatic monocarboxylic					
Formic	27.2	27.2	20 EtOH–80 Et₂O	1.76×10^{-4}	(27)
Acetic	22.2	...	Et₂O	1.75×10^{-5}	(27)
Propionic	18.8	18.4	20 EtOH–180 Et₂O	1.4×10^{-5}	(15, 27)
n-Butyric	16.3	15.6	5 EtOH–300 Et₂O	1.4×10^{-5}	(16, 27)
Isobutyric	16.3	16.2	5 EtOH–300 Et₂O	1.5×10^{-5}	(27)
n-Valeric	14.4	14.2	5 EtOH–300 Et₂O	1.6×10^{-5}	(27)
Isovaleric	14.4	9.9	5 EtOH–300 Et₂O	1.7×10^{-5}	(16, 27)
Pivalic	14.4	13.6	5 EtOH–300 Et₂O	8.9×10^{-6}	b
Caproic	12.9	12.8	5 EtOH–300 Et₂O	1.32×10^{-5}	(16, 27)
Heptylic	11.6	11.5	5 EtOH–300 Et₂O	1.28×10^{-5}	(11, 27)
Caprylic	10.6	10.5	5 EtOH–300 Et₂O	1.27×10^{-5}	(11, 19, 27)
Pelargonic	9.8	9.6	5 EtOH–300 Et₂O	1.11×10^{-5}	(11, 27)
Capric	9.1	8.6	5 EtOH–300 Et₂O	...	(11)
Lauric	7.9	7.4	5 EtOH–300 Et₂O	...	(11, 19)
Myristic	7.0	6.6	5 EtOH–300 Et₂O	...	(11, 19)
Palmitic	6.3	5.4	5 EtOH–300 Et₂O	...	(11, 17)
Stearic	5.7	5.3	5 EtOH–300 Et₂O	1.7×10^{-6}	(11, 17)
Aliphatic dicarboxylic					
Malonic	24.7	25.1	Et₂O	1.6×10^{-3}	(15)
Succinic	22.4	22.6	150 EtOH–150 Et₂O	6.6×10^{-5} 2.8×10^{-6}	(15, 16)
Glutaric	20.5	20.4	5 EtOH–150 Et₂O	4.7×10^{-5} 5.34×10^{-6}	(16)
Adipic	18.9	18.8	150 EtOH–150 Et₂O	3.90×10^{-5} 5.29×10^{-6}	(16)
Pimelic	17.5	17.2	Et₂O	3.33×10^{-5} 4.87×10^{-6}	(16)
Suberic	16.4	16.5	Et₂O	2.98×10^{-5} 4.71×10^{-6}	(16)
Azeleic	15.3	14.8	Et₂O	2.82×10^{-5} 4.65×10^{-6}	(2, 16)
Sebacic	14.4	13.8	Et₂O	2.34×10^{-5} 2.55×10^{-6}	(16)
Monoethyl esters of aliphatic dicarboxylic					
Oxalic	12.6	12.7	Et₂O	...	...d
Malonic	11.4	12.5	Et₂O	...	...d
Succinic	10.4	11.2	Et₂O^d	...	...d
Adipic	8.9	9.0	Et₂O	...	...d
Sebacic	6.9	7.4	Et₂O	...	...d
Miscellaneous					
Benzoic	12.3	12.2	5 EtOH–300 Et₂O	6.3×10^{-5}	(15)
p-Aminobenzoic	11.1	11.0	5 EtOH–300 Et₂O	...	(18)
Anthranilic	11.1	10.8	Et₂O	...	(18)
Cyclohexanecarboxylic	11.8	11.7	5 EtOH–300 Et₂O	...	...b
p-Hydroxybenzoic	11.0	10.7	Et₂O	...	(17)
Salicylic	11.0	10.6	Et₂O	1.06×10^{-3}	(17)
Acetylsalicylic	8.6	11.0	Et₂O	...	(26)
Sulfosalicylic	7.2	12.7	Et₂O	...	b
Picolinic	12.2	12.4	200 EtOH–100 Et₂O	3×10^{-6}	(24)
Aconitic	22.7	21.2	50 EtOH–250 Et₂O	...	(17)
Itaconic	20.8	20.8	25 EtOH–275 Et₂O	1.5×10^{-4} 2.8×10^{-6}	(16)
Fumaric	22.7	22.6	100 EtOH–200 Et₂O	1×10^{-3} 3×10^{-5}	...b
Maleic	22.7	21.7	10 EtOH–290 Et₂O	1.5×10^{-2} 2.6×10^{-7}	(10)
Sorbic	13.2	13.2	25 EtOH–275 Et₂O	...	...b
Crotonic	16.5	15.8	Et₂O	2.0×10^{-5}	(16)
Tartaric	18.5	18.7	125 EtOH–175 Et₂O	1.1×10^{-3} 6.9×10^{-5}	(15)
Malic	20.3	19.9	100 EtOH–200 Et₂O	4×10^{-4} 9×10^{-6}	(9, 15)
Citric	21.0	13.0	100 EtOH–200 Et₂O	8×10^{-4} 1.8×10^{-5} 4×10^{-6}	(8)
Lactic	15.9	9.2	50 EtOH–300 Et₂O	1.55×10^{-4}	(3)
Hippuric	8.7	8.8	110 EtOH–190 Et₂O	2.3×10^{-4}	(17)

^a Standard micro-Kjeldahl distillation and titration.

^b Typical synthesis: To 10 grams of 98–100% formic acid was added 20 ml. of 95% ethyl alcohol plus 80 ml. of anhydrous ether. This mixture was stirred at room temperature and anhydrous ammonia was bubbled in for 1 hour. The crystalline product was filtered through fritted glass under an ammonia atmosphere, washed with dry ether, dried in a stream of ammonia, then stored under reduced pressure over potassium hydroxide pellets.

^c From literature.

^d Typical synthesis: To 20 grams of diethyl succinate was added 2.6 grams of potassium hydroxide in 60 ml. of 95% ethyl alcohol; the solution was held at 70° C. for 24 hours, then flooded with water and acidified with concentrated sulfuric acid. The monoethyl-succinate was extracted into diethyl ether, dried over sodium sulfate, and distilled (b.p. 120–23° C./2 mm.). Anhydrous ammonia was passed for ¹/₂ hour into a solution of 5 grams of this half-ester in 300 ml. of anhydrous diethyl ether. The resulting very hygroscopic salt was filtered, dried, and stored as above.

Table III. Ammonia Vapor Concentration Data for Dry Ammonium Salts in Air at 25.0° ● 0.1° C.

Salt	Absorbance[a] Ammonium acid[b]	Parent acid[c]	Ammonia Vapor over Salt, Mg./L. If undissociated[d]	If dissociated[e]
Aliphatic monocarboxylic				
Formate	0.0140	0.5850	0.13	...f
Propionate	0.1759	0.0330	1.08	0.90
n-Valerate	0.3780	...	2.32	...
Isovalerate	0.0870	0.0240	0.53	0.37
Caproate	0.2435	Nil	1.50	1.50
Heptylate	0.1247	0.1085	0.77	0.12
Caprylate	0.1895	0.0778	1.15	0.70
Pelargonate	0.2371	Nil	1.46	1.46
Caprate	0.2950	0.0410	1.81	1.55
Laurate	0.3266	0.0105	2.01	1.96
Myristate	0.2428	0.0065	1.50	1.42
Palmitate	0.2452	0.0250	1.51	1.37
Stearate	0.1952	0.0010	1.21	1.20
Aliphatic dicarboxylic				
Malonate	0.0030	0.0210	<0.10	...f
Succinate	0.0856	0.0120	0.51	0.43
Glutarate	0.1088	0.0080	0.66	0.62
Adipate	0.0550	Nil	0.35	0.35
Pimelate	0.5240	0.0025	3.28	3.27
Suberate	0.0236	Nil	0.17	0.17
Azelate	0.4710	Nil	2.95	2.95
Sebacate	0.0964	Nil	0.59	0.59
Monoethyl esters of aliphatic dicarboxylic				
Malonate	0.0220	...	0.17	...
Sebacate	0.0680	...	0.40	...
Miscellaneous				
Benzoate	0.0090	0.0350	0.12	...f
p-Aminobenzoate	0.0285	Nil	0.19	0.19
Cyclohexanecarboxylate	0.0668	0.0976	0.39	...f

a Corrected for cell blank.

b Each salt was dried over potassium hydroxide pellets under reduced pressure in an ammonia atmosphere. Duplicate 2-mg. quantities were placed in 1-cm. sealed silica cells, incubated at 25.0° ± 0.1° C. for 48 hours over potassium hydroxide pellets under reduced pressure and with all stoppers removed. Ultraviolet absorption of vapors in the resealed cells was recorded from 196 to 206 mμ in a Beckman Model DUS spectrophotometer (4) with cell compartment thermostated at 25.0 ± 0.1° C. Inflection point at 204.3 mμ was utilized in calculations.

c Eastman white label acids without further purification, equilibrated and read in 1-cm. cells as above. The carboxyl group also absorbs at 204.3 mμ (6).

d Values assume absorption by ammonia only.

e Values corrected for saturation by vapors of parent acid.

f High vapor tension of parent acid makes calculation meaningless.

Table IV. Desiccant and Deliquescent Materials Evaluated

Material	Wt. % of Standard Pellet[a]	Material	Wt. % of Standard Pellet[a]
Magnesium sulfate[b]	13	Gran. sugar	10
	7		5
Calcium chloride[b]	10	Powd. sugar	10
	5		5
Diammonium succinate	10	Gelatin	10
	5		
Glycerol	5	Sorbitol	10
Ammonium acetate	5		5

a Proportional composition. $(NH_4)_2SO_4$, 24.4%; Na_2CO_3, 18.3%; talc, 5.8%; 30-to-70 mixture of powdered and granular bentonites, to 100%.

b Anhydrous salt.

pellets or granules, natures and proportions of excipients and exfoliants, and inclusion of accessory hygroscopic materials. The amount of ammonia in-package could thereby be regulated by the number of pellets.

Biological evaluations (13) of many metathetical mixtures with the blue-green molds demonstrated that under laboratory conditions of bioassay there was considerable permissible latitude in composition. In general, stoichiometric quantities of alkaline agents and ammonium salts were used to ensure quantitative generation of ammonia, as free alkali can cause contact injury to fruit rind.

Many considerations—i.e., availability, cost, toxicology, and physical properties favorable to optimum behavior when pelletized into ammonia generators, in addition to biological performance (12, 13)—influenced the final choice of components for the large-scale commercial production of pellets. Among the alkaline agents, calcium oxide caused uncontrollably rapid evolution of ammonia with consequent injury to the fruits. The inexpensive granular magnesium oxide was too inert to be effective, whereas the very effective powdered forms were considerably more expensive than other equally satisfactory alkaline agents. Sodium sesquicarbonate (20) performed as well as soda ash in limited tests (13), but soda ash was chosen as the standard alkaline agent because of the extensive exploratory background established for it. Formulations containing ammonium chloride became objectionably wet. The hydrolytic reaction of ammonium biborate to yield ammonia and ammonium pentaborate is very promising, but elaborate evaluations of formulations containing this material are contingent upon the clarification of the toxicological status of boron-containing compounds in contact with foodstuffs.

Modification of Ammonia Generators for Specific Purposes

Formulations for ammonia generators can be designed for a specific commodity under specific environmental conditions. As ammonia gas is fungicidal to several mold organisms (12), it may be anticipated that the ammonia treatment will be applied to commodities other than citrus fruits. These other commodities might well require a fumigation pattern different from that required by citrus fruits. The environmental conditions within citrus cartons described in Table I are not always maintained during shipment. Lemons are usually precooled before shipment, and thereby receive a decreased initial fumigation with ammonia from the temperature-dependent generators described in this paper. Other factors which affect the environment, and thus the ammonia evolution pattern, are the type of packaging material, commodity, and shipping conditions and routes. Thus, for each specific fumigation condition, a modified formulation may be needed.

Exfoliants. Exfoliants in a formulation compressed into pellets cause sloughing of the pellet surface and continuous exposure of fresh areas of the pellet to the action of moisture. This steady rate of ammonia evolution in a pellet-type generator is so desirable that experimental proportions of exfoliants were varied only between 40 and 60% of the total weight of a pellet. Of all the exfoliants tested, a 30-to-70 mixture of powdered and granular bentonite was chosen for inclusion in formulations because of its superior performance during both manufacture and use compared to formulations containing either type alone. Attaclay, fuller's earth, and Celite infusorial earth (both 30-to-60 mesh)

performed well in limited tests and could probably be substituted for the bentonite mixture.

Moisture Attractants. The addition of a number of moisture attractants, including both desiccant and deliquescent materials, to a soda ash–ammonium sulfate formulation was explored because they might attract the water necessary for efficient chemical reaction and thus produce a less temperature-dependent formulation. Candidate materials (Table IV) tested ranged from weak to strong moisture attractants.

These experimental formulations were tested (13) for biological efficiency under simulated shipping conditions with both lemons and Valencia oranges by the method of Roistacher, Klotz, and Eaks (22). The moisture attractants which proved most effective from a biological standpoint were 10% calcium chloride and 10% diammonium succinate.

Laboratory Evaluation of Ammonia Generators

Because variation in any biological assay requires adequate replication and statistical evaluation, laboratory methods of evaluation of the performance of experimental ammonia generator formulations using physical and chemical tests were devised. Thus, the formulations listed in Table V were evaluated by (A) the passing of air at 50° F. and 100% relative humidity over the formulation at an arbitrary rate of 1.7 liters per hour, as in Figure 2, and periodically titrating the ammonia trapped in the boric acid solution, using bromocresol green–methyl red mixed indicator solution; (B) the periodic removal of units of a formulation from vented cartons of fruits, under simulated shipping conditions (23), for Kjeldahl analysis of resident ammonia; (C) the periodic spectrophotometric (4) determination of ammonia in the air surrounding the fruit in nonvented cartons of treated lemons as shown in Figure 3 for both room temperature conditions and simulated commercial icing conditions (see Table VI); and (D) miscellaneous evaluation procedures.

Results. The results from the three major methods are graphically presented for each formulation in Figures 4 to 7. The graphed values for Type C curves (4) are in parts per million-hours, which is the ammonia index (13, 22, 23) as determined by the area under the plot of parts per million of ammonia found in a carton *vs.* hours. In Table VI are presented cumulative ammonia-index values for Method C-type evaluation at room temperature as well as under simulated commercial icing conditions.

Discussion. From Figures 4 to 7 it is evident that several types of evolution patterns are possible. Use of any of these formulations or a combination of

Table V. Composition of Pellet[a] Formulations Extensively Tested in Commercial Shipments

Ingredient	Formulation, Grams/2.07-Gram Pellet			
	I	II	III	IV
Ammonium sulfate	0.50	0.50	0.50	...
Ammonium carbonate	...	...	...	0.43
Diammonium succinate	...	...	0.20	...
Soda ash	0.38	0.38	0.38	...
Bentonite powder	0.30	0.23	0.23	0.43
Bentonite granular	0.69	0.56	0.56	1.01
Talc	0.20	0.20	0.20	0.20
Calcium chloride	...	0.20	...	...

[a] Each pellet was 3/4 inch in diameter, 1/8 inch thick, and weighed 2.07 grams.

Table VI. Accumulative Ammonia Index Values (13, 22, 23) for Experimental Ammonia Generator Formulations

Formula	Conditions	Elapsed Days				
		1	3	6	11	16
I	Simulated commercial icing[a]	288	504	644	1,350	7,277
	Room temperature[b]	384	912	1,680	5,664	6,384
II	Simulated commercial icing[a]	432	2,074	3,658	5,578	7,402
	Room temperature[b]	1,152	7,950	10,350	12,654	13,998
III	Simulated commercial icing[a]	528	2,314	6,346	9,130	11,626
	Room temperature[b]	2,016	11,520	14,832	16,704	17,712
IV	Simulated commercial icing[a]	2,304	3,186	4,626	5,394	6,498
	Room temperature[b]	2,808	7,008	8.352	10,176	11,712

[a] When carton was placed in iced refrigerator, temperature of air around fruit dropped from initial 68° F. to 50° F. in 2 days. It remained at 50° F. during 9-day icing period, then rose to 68° F. in 2 days and remained at that temperature to end of test.
[b] Varied from 68° to 76° F.

them should make possible almost any evolution pattern desired for a particular purpose or environment.

According to Roistacher *et al.* (22, 23), with citrus fruits the product of the concentration of ammonia in air and the time in hours of ammonia treatment results in a value which may be correlated
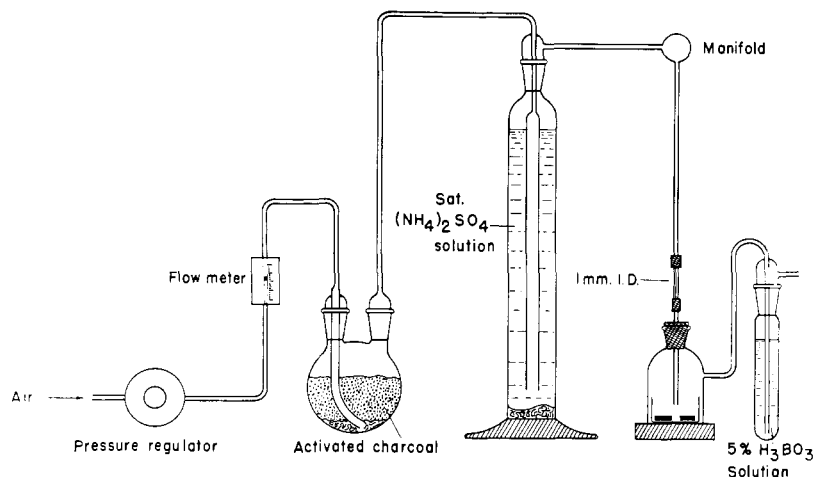

Figure 2. Apparatus used for type-A "bubbler-technique" elaboration of evolution pattern of experimental ammonia-generating formulations
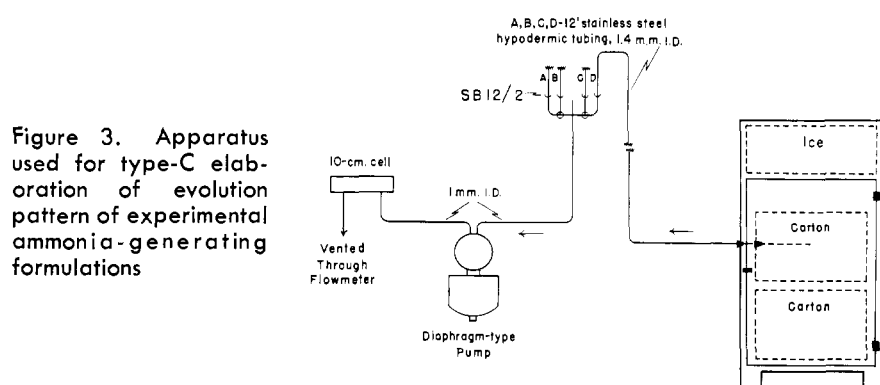
Figure 3. Apparatus used for type-C elaboration of evolution pattern of experimental ammonia-generating formulations
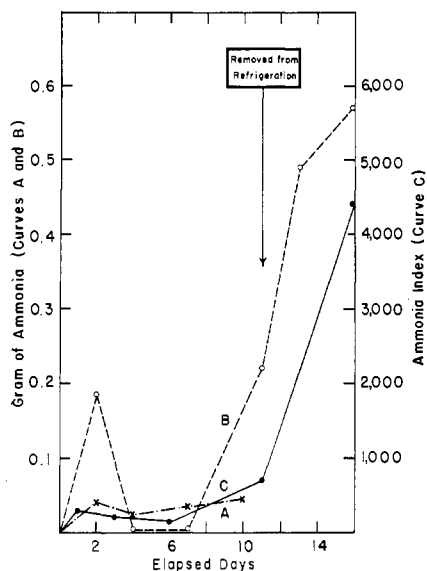
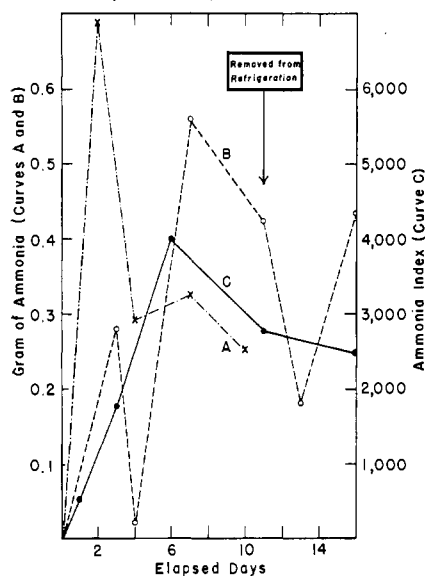Figure 4. Ammonia evolution pattern for soda ash—ammonium sulfate (formulation I, Table V)

Figure 5. Ammonia evolution pattern for the soda ash—ammonium sulfate formulation containing 10% calcium chloride (formulation II, Table V)

Figure 8. Ammonia evolution pattern for soda ash—ammonium sulfate (formulation I)

○ Placebo-weight method
● Method B

Figure 6. Ammonia evolution pattern for the soda ash—ammonium sulfate formulation containing 10% diammonium succinate (formulation III, Table V)

Figure 7. Ammonia evolution pattern for ammonium carbonate (formulation IV, Table V)

A, B, and C refer to evaluation methods A, B, and C

the ammonia-index values which may be calculated from the results are useful in predicting effectiveness. Any reliable analytical method for determining the concentration of ammonia may be used.

Several miscellaneous methods for evaluating ammonia generator formulations were used for early exploratory types of generators. For example, determination of evolved ammonia was established by direct weighing of the difference between both an ammonia generator formulation and a placebo formulation (which did not contain an ammonium salt) kept at constant temperature and constant relative humidity (Figure 8). Also, moist air passed over a formulation in either of two apparatuses (6) was examined for ammonia content by direct spectrophotometric means. Probably the most valuable of these miscellaneous methods is the use of the apparatus (6) incorporating the Beckman DK-2 ratio-recording spectrophotometer; in Figure 9 is a typical chart resulting from this type of evaluation. Use of this apparatus under standardized conditions provided a quick, accurate picture of the ability of a formulation to evolve ammonia under various conditions of temperature and of relative humidity.

Present Commercial Ammonia Generator

As a result of tests and criteria discussed above and applied to over 100 candidate formulations, the pellet formulations listed in Table V were chosen for initial testing under commercial shipping conditions. The decay-preventing results of these tests will be reported (13).

These biological tests as well as considerations of fruit injury indicated that formulations containing soda ash in excess of stoichiometric ratios with respect to ammonium sulfate were not superior to those in which equimolar ratios were used. Consequently, a 1-to-1 molar ratio of reactants was chosen for the commercial formulations. Because formulation I (Table V) has been studied on a vastly greater scale than the others, its manufacture is discussed in some detail.

with fungicidal effectiveness as well as with fruit injury. This ammonia-index value for maximum biological effectiveness with a minimum of fruit injury should be between 300 and 6000 p.p.m.-hours, as determined by a Nessler's test (22, 23). This effectiveness range was investigated (22) with the Nessler's test which yields consistently low values compared to the spectrophotometric method used in this work; the actual effectiveness range would therefore consist of somewhat higher values if determined spectrophotometrically. The ammonia-index values in Table VI demonstrate the gross effect of temperature upon the rates and evolution patterns of the formulations and thus upon their effectiveness. According to these values, all formula-
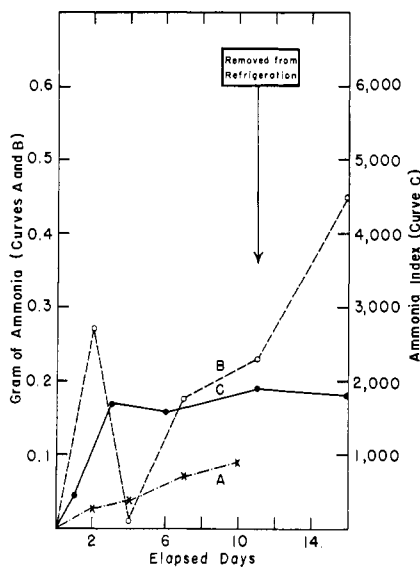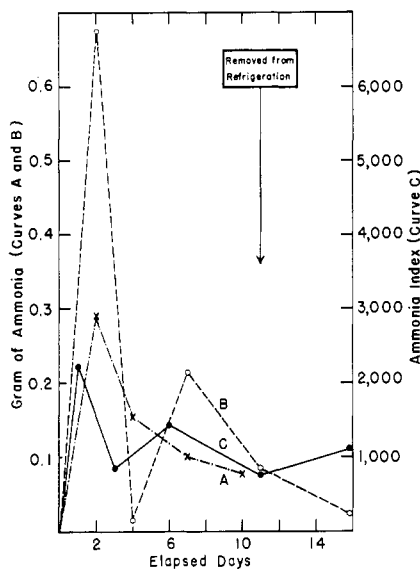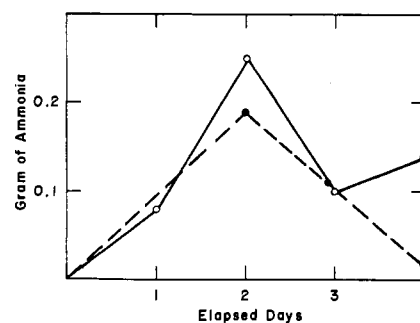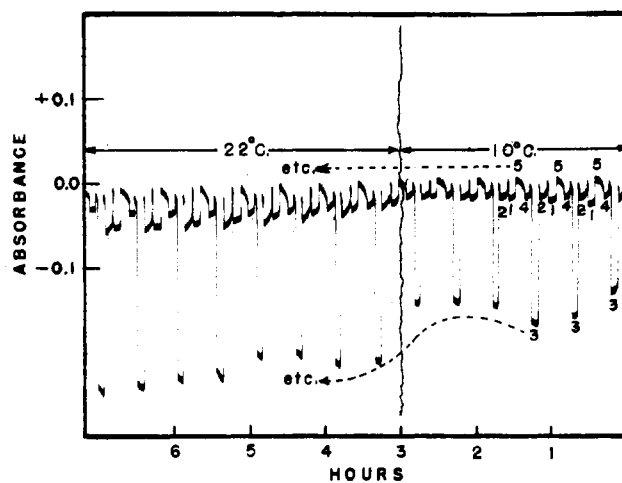
tions represented would be effective for citrus fruits under simulated commercial icing conditions; all but the standard formulation I (Table V) would injure fruits at room temperature.

The three major methods used for evaluation of formulations provide essentially equivalent evolution patterns (Figures 4 to 7). For the present purposes, Method A, the "bubbler-technique" method, is too inflexible to be adapted easily to the many variables in shipping conditions. Method B, the "mat-analysis" method, is too expensive in that it requires large quantities of fruit. Method C, which establishes the actual parts per million concentration of ammonia in the carton atmosphere under conditions of commercial use, gives the most information, as

Figure 9. Typical evolution record of four tableted exploratory ammonia generator formulations each containing 2 grams of equivalent ammonia

1. 10.4 grams of a mixture of 0.5 gram of diammonium succinate, 0.30 gram of ammonium sulfate, 0.12 gram of magnesium oxide, and 0.08 gram of magnesium sulfate per tablet
2. 16.7 grams of a mixture of 1.5 grams of diammonium succinate and 1.5 grams

of bentonite per tablet
3. 10.7 grams of a mixture of 0.5 gram of diammonium succinate and 1.5 grams of magnesium oxide per tablet
4. 9.0 grams of diammonium succinate
5. Air purge (5)

Satisfactory operation of commercial pellet-making machinery requires that material to be pelletized be free flowing without becoming tacky during the process, and not unduly abrasive to the dies. Addition of a lubricant to the active ingredients in the formulation was essential (25) to minimize wear of the dies. An ideal lubricant would lubricate freely, add strength to the pellet, and not retard moisture attraction by the pellet. Talc, graphite, and magnesium and calcium stearates were evaluated (25). Of these, talc most nearly represented the ideal lubricant. Many lots of formulation I were made to contain 0.2% actual graphite in addition to the talc, but they were more variable in performance than the slightly weaker, slightly more abrasive pellets without graphite.

An additional consideration in this problem was the need to minimize the reaction and resultant evolution of ammonia caused by small quantities of water present in the ingredients of the pellet as well as by the heat and pressure of the manufacturing process. When soda ash was coated with powdered bentonite before addition of ammonium sulfate to the mechanical mixer, evolution of ammonia during manufacture was not serious (25). Therefore, the exfoliant used was a mixture composed of 30% powdered ben-

Table VII. Typical Water Content of Major Materials Used to Make Pellets Commercially

Pellet[a] Material	% H_2O[b]
$(NH_4)_2SO_4$	0.08
Na_2CO_3	[c]
Talc	0.37
Bentonite	10.4

[a] Winning-Peplow, Inc., Los Angeles, Calif.
[b] By standard Karl Fischer titration.
[c] Cannot be done by Karl Fischer method.

tonite and 70% granular bentonite; the latter imparted free-flowing properties to the mixture before pelletizing.

Analyses of representative samples of the major components of formulation I by Karl Fischer titration gave the results shown in Table VII. The normal water content of bentonite appears to be tightly bound with the mineral and does not cause initial reaction of the active components. Ammonia content was not seriously diminished during storage for a year of commercial pellets of this formulation in tightly closed cans.

Since an important factor in the use of these pellets as ammonia generators was the ammonia available per pellet, analyses to establish constancy of composition during commercial manufacture were important. While the contents of a 1600-pound capacity mixer were being emptied into barrels for transport to the pellet presses, periodic samplings of the formulation were analyzed for ammonia content with results ranging from 5.26 to 6.52%, with an average of 6.05% for the 11 samples involved. Completed pellets were also analyzed periodically for ammonia content by representatively sampling a number of 1600-pound lots over 3 months; results ranged from 5.08 to 5.95%, with an average of 5.69% for the nine samples involved.

For ease in handling and for protection of treated commodities from soilage and contamination by the components of the formulation, the pellets were enclosed in mats. A typical mat was made from a sheet of nonporous, plastic-coated manila paper heat-sealed to another sheet of porous paper in such a manner that 9 pellets per mat were enclosed in 3 pockets of 3 pellets each; its over-all dimensions were 8.0 × 11.2 cm. Water vapor can pass inward toward the pellets and ammonia outward toward the fruit through the pervious face of the mat.

The nonporous side can be faced toward the fruit to minimize fruit burn resulting from high ammonia concentrations in a localized area adjacent to the mat.

This system of pelletizing and packaging in convenient units provides flexibility in the amount of available ammonia to be introduced into a carton of fruit. The sizes and number of pellets per mat as well as the number of mats per carton are then a matter of choice to meet a particular set of conditions applicable to a particular commodity.

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FUNGICIDES FOR PACKAGED FOODS

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

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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 (11), 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