A brief statement of the use of ammonium compounds in food processing: Nutriphysiological perspectives.

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Introduction

1. Introduction
    a. nomenclature of ammonium hydroxide
    b. historical usage of ammonia in the food industry
    c. disappearance data of ammonia and ammonium hydroxide
    d. US government status
    e. countries (First world) that have established GRAS status for ammonium hydroxide

The atmosphere of the earth is composed primarily of elemental nitrogen. This form of nitrogen is not available for life to use in metabolic processes rather it must be converted or fixed into a reduced or oxygenated form. Ammonia (NH_3), nitrate (NO_3^+) and nitrite (NO_2) are amendable to use in life with nitrate being especially useful for plants as fertilizer. The conversion of ammonia to the oxygenated forms occurs through nitrifying bacteria and goes through the intermediate of nitrite. Nitrogen cycling is one of the most nutrient cycles important as it converts elemental nitrogen into forms that are useable by life.

Historically nitrate was derived from guano sources. In the early 1900's, Haber developed a process for chemical conversion of elemental nitrogen into ammonia. The Haber process ammonia could then easily be converted into nitrate for fertilizers or used directly as anhydrous or ammonium salts. Softened and de-ionized water is used to produce food grade (FCC) grade ammonium hydroxide (aka aqua ammonia). Ammonium salts and other hardness compounds that precipitate out of solution are removed by filtration. Impurities in the water result in other ammonium salts and other compounds being produced which then requires them to be removed.

Aroma threshold of ammonia has been reported from 0.6 (Stopford et al., 1988) to 1.5 ppm (WHO, 2002) while taste threshold in pure water is 35 ppm. If added to water, the anhydrous form becomes the hydroxide. When converted to other forms such as salts or hydroxide, ammonia maintains a milder aroma and the health aspects of the compound change significantly.

When added to water, ammonia readily forms the hydroxide form - ammonium hydroxide. In this state the ammonia does not have the aroma impact as the straight compound. Aqueous forms of ammonia are also known as ammonia solution, aqua ammonia, and liquid ammonia. It is available in a variety of concentrations (1-30%) in this form. The pH and the relative density of the ammonium solution will vary with concentration. As concentration increases, pH will increase to 13.5 at 30% concentration. Density varies inversely with concentration eg less dense at higher concentrations. This feature allows measurement of concentration by calibrated hydrometer. Ammonium chloride, ammonium sulfate, ammonium hydroxide, and other salts of ammonium are used in a variety of food industries for a several purposes including facilitating browning reactions, acid control, leavening agent, and as an antimicrobial. Table 1 shows the approved usage rate and reason for use in a selection of food products.

Table 1. Maximum allowed levels of select ammonium compounds in specific processed foods.

	Ammonium Source Recommended Approved Usage Rate (%)					
	Ammonium Chloride	Ammonium Carbonate	Ammonium Bicarbonate	Ammonium Hydroxide	Ammonium Phosphate	
Food					Mono	Di
baked goods	0.001	2.0	0.04-3.2	0.6-0.8	0.01	1.1
Grains			0.04-3.2			
Condiments/relishes	0.8					0.012
Gelatins/puddings		2.0		0.6-0.8		
Cheeses				0.6-0.8		
Foods			0.04-3.2	GMP*		
Snack Foods			0.04-3.2			
Reconstituted vegetables			0.04-3.2			

*Upper limit is not defined, GMP status allows use that would be consistent with a GRAS status additive. Note that 1.0 % is equivalent to 10,000 parts per million.
Source: Agency for Toxic Substances and Disease Registry, CDC, TP126 – Ammonia.

The FDA (1973, 2003) determined that concentrations of ammonia and ammonium compounds normally present in food do not suggest a health risk; ammonia and ammonium ions are recognized to be integral components of normal metabolic processes. However, some restrictions have been placed on levels of ammonium salts allowable in processed of foods. Maximum allowable levels in processed foods are as follows are given in Table 1. Food ingestion can also lead to an exposure to ammonia, primarily due to the use of various ammonium salts as food stabilizers; the estimated exposure from these food additives is 18 mg/day.

The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Program. The main purposes of this Program are protecting health of the consumers and ensuring fair trade practices in the food trade, and promoting coordination of all food standards work undertaken by international governmental and non-governmental organizations. Codex standards are based on scientific advice as provided by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). JECFA is an international scientific expert committee that is administered jointly by the Food and Agriculture Organization of the United Nations FAO and the World Health Organization WHO. Ammonium hydroxide has been given the INCHEM number INS 527.

The conclusion of JECFA is that ammonium hydroxide can be used within good manufacturing practices context. The list of approved foods for which ammonium hydroxide may be used in is given in Table 2.

There are relatively few countries that have not adopted the recommendations of JEFCA. Ammonium hydroxide is approved for use in most countries of the world including the EU (E 527), Australia/New Zealand (approved as a processing aid), US, and most others.

Table 2. Codex Alimentarius Commission Findings on Ammonium Hydroxide
Ammonium Hydroxide is included in the Codex Alimentarius and as such may be used in the following foods under the conditions of good manufacturing practices (GMP) as outlined in the Preamble of the Codex GSFA.

Number	Food Category
Dairy	
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)
01.3	Condensed milk and analogues (plain)
01.4.3	Clotted cream (plain)
01.4.4	Cream analogues
01.5	Milk powder and cream powder and powder analogues (plain)
01.6	Cheese and analogues
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)
01.8	Whey and whey products, excluding whey cheeses
Fats/oils	
02.2.1.2	Margarine and similar products
02.2.1.3	Blends of butter and margarine
02.2.2	Emulsions containing less than 80% fat
02.3	Fat emulsions maily of type oil-in-water, including mixed and/or flavoured products based on fat emulsions
02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7
Frozen desserts	
03.0	Edible ices, including sherbet and sorbet

Fruits/vegetables

04.1.2	Processed fruit
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine, or soy sauce
04.2.2.4	Canned or bottled (pasteurized) or retort pouch vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds
04.2.2.5	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter)
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g., vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5
04.2.2.8	Cooked or fried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds

Confections

05.0	Confectionery

Cereals/milled products

06.3	Breakfast cereals, including rolled oats
06.4.3	Pre-cooked pastas and noodles and like products
06.5	Cereal and starch based desserts (e.g., rice pudding, tapioca pudding)
06.6	Batters (e.g., for breading or batters for fish or poultry)
06.7	Pre-cooked or processed rice products, including rice cakes (Oriental type only)
06.8	Soybean products (excluding soybean products of food category 12.9 and fermented soybean products of food category 12.10)

Bakery

07.0	Bakery wares

Meats

08.2	Processed meat, poultry, and game products in whole pieces or cuts
08.3	Processed comminuted meat, poultry, and game products
08.4	Edible casings (e.g., sausage casings)

Fish/crustaceans

09.3	Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms
09.4	Fully preserved, including canned or fermented fish and fish products, including mollusks, crustaceans, and echinoderms

Egg Products

10.2.3	Dried and/or heat coagulated egg products
10.3	Preserved eggs, including alkaline, salted, and canned eggs
10.4	Egg-based desserts (e.g., custard)

Sweeteners

11.6	Table-top sweeteners, including those containing high-intensity sweeteners

Seasonings/condiments/sauces/broths

12.2.2	Seasonings and condiments
12.3	Vinegars
12.4	Mustards
12.5	Soups and broths
12.6	Sauces and like products
12.7	Salads (e.g., macaroni salad, potato salad) and sandwich spreads excluding cocoa- and nut-based spreads of food categories 04.2.2.5 and 05.1.3
12.8	Yeast and like products
12.9	Protein products
12.10	Fermented soybean products

Dietetic foods

13.3	Dietetic foods intended for special medical purposes (excluding products of food category 13.1)
13.4	Dietetic formulae for slimming purposes and weight reduction
13.5	Dietetic foods (e.g., supplementary foods for dietary use) excluding products of food categories 13.1 - 13.4 and 13.6
13.6	Food supplements

Beverages

14.1.1.2	Table waters and soda waters
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks
14.2.1	Beer and malt beverages
14.2.2	Cider and perry
14.2.4	Wines (other than grape)
14.2.5	Mead
14.2.6	Distilled spirituous beverages containing more than 15% alcohol
14.2.7	Aromatized alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)

Ready-to-eat savories

15.0	Ready-to-eat savouries

Composite foods

16.0	Composite foods - foods that could not be placed in categories 01 - 15

Note: Unless otherwise specified, these provisions apply to the food category indicated (e.g. Dairy), as well as to all subcategories of that category (e.g. Cheese, Ripened Cheese, etc.).

Source: http://www.codexalimentarius.net/gsfaonline/additives/details.html?id=380

Chemical reactions involving ammonia ion

a. formation of ammonium hydroxide
b. reactions with acids
c. reactions with organic molecules such as proteins, carbohydrates, and fats

$NH_3(aq) + H_2O(l) \leftrightarrow NH_4^+(aq) + OH^-(aq)$ aqueous ammonia (ammonium hydroxide)

The addition of ammonium to water results in the production of the ammonium ion (NH_4^+) and a hydroxide ion. The actual analysis of water for ammonium hydroxide results in the calculation of ammonia as a percentage of the solution. Hence the name aqueous ammonia is probably more correct than ammonium hydroxide. Aqueous ammonia may be generated in solution from a variety of sources that include the release of anhydrous ammonia to water and the dissociation of ammonium salts in water. The solution is readily reactive with acids resulting in production of ammonium salts such as ammonium chloride, ammonium sulfate, and others. Toxicity of these ammonium salts is discussed below. Several of these salts are used in the food processing industry to generate ammonia water/ammonia. In general, these compounds breakdown to give off ammonia during a thermal process.

The reaction of most molecules with ammonium hydroxide involves the alkaline nature of the compound or the formation of a reactive amine. For example, ammonia reacts with sugars in the Maillard reaction with the end product being brown or caramel colors. This is a traditional manufacturing process for commercial caramel colorants used in the food industry. Other uses of ammonium hydroxide include the control or neutralization of acid components in foods. In addition, ammonium ion appears to have a significant antimicrobial effect aside from the pH change that is effected when added to meat products (Gupta et al., 1988).

Nutri-physiological aspects of ammonium ingestion

Research has been done on the feeding of the ammonium ion in various forms to animals including humans. These studies have shown little to modest affects depending upon the model organism. The metabolism of ammonia in the body has been well researched. The end result of these two areas of research is that ammonium hydroxide has been considered a safe ingredient when used according to Good Manufacturing Practices (Codex General Standard for Food

Additives, Standard 192, revised 2006). It is useful to note that ammonia is readily absorbed by the body and is produced in moderately high quantities ca. 4 – 8 grams/day in the gut of humans. It has been estimated that up to 17 grams of ammonia are produced in humans daily (WHO, 1986). Ammonia is excreted primarily as urea and urinary ammonium compounds through the kidneys.

As stated in the Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry Toxicological Profile for ammonia, oral minimal risk levels were not derived for ammonia because of the lack of data and the fact that when reported the effect of the attendant anion is not considered. The only human acute oral studies available were case reports with no exposure levels (ASTDR, TP126, 2004). When rats were fed a diet composed of 20% ammonium acetate and water containing 5 mM ammonium acetate for 90 days, no impact on key proteins and blood protein or ammonia levels were noted (Bodega et al., 1993). The authors indicated that after an initial change in blood values, adaptation resulted in returns of all parameters to normal. It should be noted that WHO (WHO, 1986) estimates that ingested ammonia (as food additives) is 18 mg/day above and beyond the amount found in foods.

Intentional human oral exposure studies that have lasted for longer than a week or so have not been done and reported deaths from oral exposure are very rare. However based upon animal models, a relative lethal dose may be calculated for ammonia. Lethal dosage in animals appears to be in the 3-5 g/kg/day range as ammonium sulfate (WHO, 1986). For a 50 or 70 kg human these would equate to 150-250 g/day and 210-350 g/day, respectively. These amounts are inconceivable ingestion rates of one quarter to over a half pound of ammonia compounds per day. Using ammonium carbonate as an example, even at maximum exposure levels of 32,000 ppm as allowed in some foods, you would need to consume more than 10 lbs of that particular food in order to ingest one quarter pound of the ammonium carbonate containing compound.

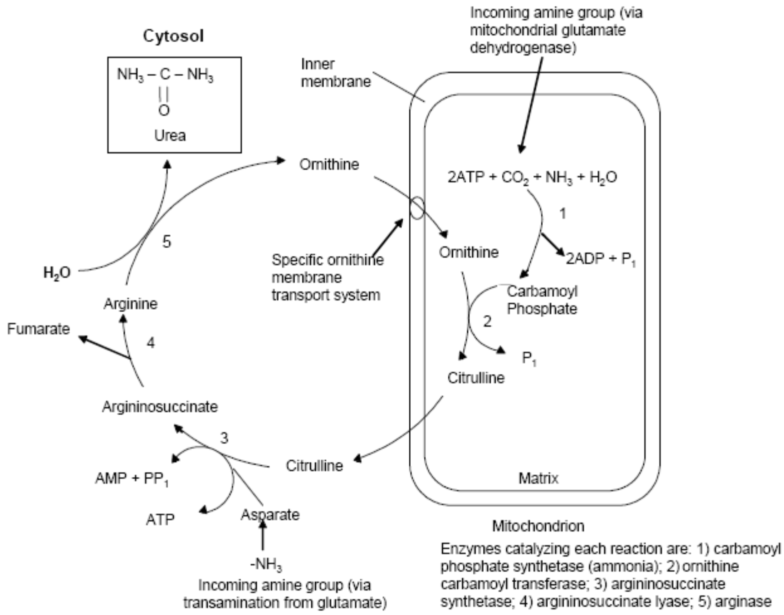
So, the amount of excess ammonia (i.e., over and above the amount normally produced in the body) that can be safely ingested and assimilated is difficult to define. In short-term (28 - 90 days) studies carried out on rats and pigs, no adverse effects were reported at higher levels of ammonia intake (75 - 545 mg NH_3/kg body weight per day) in the form of sulfamate, phosphate, citrate, or chloride (WHO, 1986). These ranges are in the 4 – 27 g/day for 50 kg person or 5 – 38 g/day for a 70 kg person.

Ammonium has not been shown to be carcinogenic. Mutagenic activity of ammonium has only been shown at very high levels in cell or other models.

The principal source of ammonia in the body is the deamination through oxidation of amino acids, in particular, glutamic acid. Glutamate dehydrogenase is the key enzyme in this production with lesser amounts produced through the deamination of other amino acids. Ammonia thus produced used or excreted through a variety of biochemical pathways. These pathways include: reversal of glutamate dehydrogenase results in synthesis of amino acids; the production of the nitrogenous amino acids glutamine and asparagine; and lastly synthesis of carbamoyl phosphate, the key intermediate in arginine and pyrimidine biosynthesis. It is through pathways involving arginine hydrolysis that urea is produced. Urea serves as the terminal molecule in nitrogen metabolism in many animals including humans.

Ammonium ions are essential to metabolism of animals, plants, and microorganisms

Nitrogen cycling is a critical to life on earth. Ammonia ions play a crucial role in this cycling because of their use in all life. Basic to all life are proteins comprised of some twenty different amino acids. In plants and microorganism, most of these amino acids are synthesized from organic or inorganic nitrogen including ammonia. In humans and other animals, a significant number of amino acids are not synthesized but are considered essential and must be consumed as intact amino acids. Those amino acids that are synthesized in animals are often the result of transamination reactions involving simple carbon skeletons derived through energy production (Krebs cycle intermediates). The figure below shows the nitrogen cycle in humans (Lehninger, 1976).



Source: Lehninger 1975

Ammonia ions ($NH_4^+ + OH \leftarrow NH_4OH$) are used by gastrointestinal tract (GIT) microorganisms to produce some amino acids which are absorbed or passed on by the host. In certain models, it has been shown that the essential amino acids produced by GIT bacteria may be absorbed by and contribute to the well being of the host (reference). In humans, when protein labeled with the stable isotope ^{15}N ($^{15}NH_3^+$ amino group) were introduced directly to the large intestine, the label showed up in body proteins. The reason is that the amino acids in the protein are deaminated with the labeled ammonia taken up by through the intestine. Thus, ammonia is an important nutrient that is needed by the body to complete protein synthesis.

When humans were fed oral dosages of isotope ^{15}N ammonia, the label showed up almost immediately in blood plasma proteins. This suggests that dietary ammonia is readily absorbed and is used in amino acid synthesis. The amounts of ammonia used for these trials were in the range of 100 ppm/minute of the 6 hour trial (Patterson et al., 1995). What is interesting is that the majority of the dietary ammonia used in this study was used in amino acid synthesis rather than urea production. This indicates that the human utilizes dietary ammonia for protein synthesis selectively over transferring it to urea waste streams. This is in contrast to much earlier studies that showed approximately 70% of ingested ^{15}N ($^{15}NH_3^+$ amino group) was excreted within 6 hours after ingestion (Furst, 1969; Richard, 1975).

There have been cases of ingested ammonia intoxication. These are typically with cleansers that contain 8-12.5% ammonia on a weight basis. It is interesting that when ingested, ammonia causes both physical and metabolic problems of which the physical are more severe than the latter. Burns and blistering of the exposed tissues are the major physical manifestations of ingestion. The metabolic issues can be resolved through use of oxygen and hydration therapy. In the case of a adolescent who presented with five times the normal blood ammonia concentrations, symptoms were resolved within 48 hours of treatment with no sequential sequelae. The individual was forced to drink ammonia containing cleaning compound. In other situations where children or adults have consumed ammonia, it was the damage caused by chemical burns to the esophagus and mouth that were the primary concerns rather than the increase in blood parameters. It would appear that ammonia is a relatively poor toxin when ingested. Hence its safety as ammonium hydroxide and the GMP status it has according to FAO.

Summary

It becomes clear that ammonia is one of the major metabolites of the human body. Both humans and their constituent microorganisms produce gram quantities of ammonia that are used internally or excreted as urea or other forms. Estimates are that up to 17 grams of ammonia are ingested and/or metabolized each day by the human. The amount of ingested ammonia from additives has been estimated at less than 20 *milli*grams/day or 0.02 g. This is approximately 850 times less than the amounts produced endogenously in humans and exogenously by intestinal bacteria. Regulatory status of ammonium hydroxide is well established with the material being

considered GRAS as used under GMPs. The compound is approved for use in the United States, EU, Australia, New Zealand, and many other countries and entities.

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