Glucono delta-lactone

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
D-glucono-delta-lactone
delta-D-gluconolactone
D-glucono-1,5-lactone

Other Name:
Glucono delta-lactone
GDL

Trade Names:
GDL F5010, GDL F2500

CAS Numbers:
90-80-2

Other Codes:
E 575 (European Union)
INS 575 (Codex Alimentarius)
EINECS 202-016-5 (European Inventory Number)

Summary of Petitioned Use

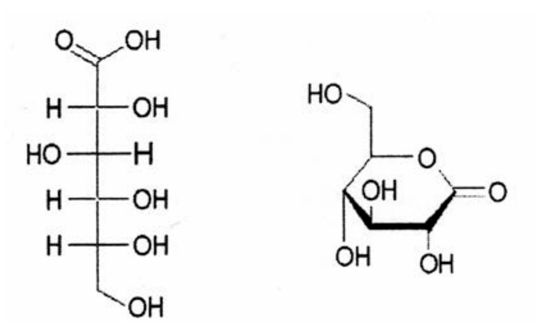
Glucono delta-lactone (GDL) is currently allowed under the National Organic Program (NOP) regulations at 7 CFR 205.605(a). It is an allowed nonsynthetic ingredient in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))," provided that it meets the annotation requirement, "production by the oxidation of D-glucose with bromine water is prohibited." It can be used in organic processing for all Generally Recognized as Safe (GRAS) applications. GDL can be added to food as a curing and pickling agent, a leavening agent, a pH control agent, and as a sequestrant (21 CFR 184.1318). It was originally petitioned for use as a tofu coagulant.

Characterization of Petitioned Substance

Composition of the Substance:

D-glucose is an aldohexose, which means it is a six-carbon sugar containing an aldehyde group. When the aldehyde group is oxidized to a carboxylic acid, D-gluconic acid is formed. The carbon adjacent to the acid group is called *alpha* (C2), the next one is *beta* (C3), the next is *gamma* (C4), and the next is *delta* (C5).

D-gluconic acid can react with itself, forming a cyclic ester called a lactone. When the reaction is between the acid group on C1 and a hydroxyl group on the *delta* (C5) carbon, the lactone is called *delta*-D-gluconolactone, D-glucono-*delta*-lactone or D-glucono-1,5-lactone (Ramachandran et al. 2006).



Gluconic Acid **Glucono Delta-Lactone**

(For the rest of this report, D-glucose will simply be called glucose, D-gluconic acid will be called gluconic acid, and *delta*-D-gluconolactone will be called glucono delta-lactone or GDL).

Source or Origin of the Substance:

Glucose is present naturally in many foods. When it reacts with oxygen in the air, some of it oxidizes to gluconic acid. Gluconic acid can be found in honey (up to 1%), wine (up to 0.25%), and in rice, meat and vinegar. (Amounts are higher in honey because the enzyme glucose oxidase is a salivary secretion of the honey bee, *Apis mellifera*, so there is some enzymatic conversion.) Wherever gluconic acid occurs in the presence of water, some of it can cyclize to glucono delta-lactone (Ramachandran et al. 2006; Wong et al. 2008).

Glucono delta-lactone is also produced industrially by a number of methods. (See Evaluation Questions 1 and 2.)

Properties of the Substance:

Table 1: Properties of glucono delta-lactone and gluconic acid (UNEP 2006)

	Glucono delta-lactone	**Gluconic acid**
Physical state	white solid	white solid
Melting point	153°C	131°C
Estimated boiling point	398.5°C	417.1°C
Density	1.68 g/ml	1.23 g/ml
Vapor pressure	2.41×10^{-9} hPa at 25°C	10.87×10^{-10} hPa at 25°C
Water solubility	590 g/liter at 25°C	1000 g/liter at 25°C
pKa	3.70	3.7
Molecular weight	178.14	196.16
Molecular formula	$C_6H_{10}O_6$	$C_6H_{12}O_7$

Specific Uses of the Substance:

Coagulant in tofu production

GDL is used as a coagulant in the production of tofu. It was introduced for this purpose in the 1980s (Lim et al. 1990). To make tofu, soybeans are soaked in water overnight, and then ground into a slurry. Water is added to obtain an optimal 10:1 ratio of water to beans. The slurry is heated or boiled or steam cooked for a short time (5-10 min); then coarse solids are filtered off, leaving soymilk with soluble protein and suspended small particles (Beddows and Wong 1987a). The soymilk produced typically contains about 10% total solids, including 4.7% protein and 2.5% fat (deMan et al. 1986). Coagulant is added to the resulting soymilk either with or without stirring. Stirring produces a firmer product. The coagulated bean curd is called tofu (Tsai et al. 1981; Beddows and Wong 1987a, b and c).

Coagulants used in tofu production include (1) chloride salts such as magnesium chloride, calcium chloride, and nigari; (2) sulfate salts such as calcium sulfate and magnesium sulfate; (3) GDL; or (4) citrus juices or lactic acid (Chang 2006; Sanjay et al. 2008). Nigari coagulant is commonly used in Japan, and is produced by removing sodium chloride from seawater, then evaporating the solution. Nigari is mostly composed of magnesium chloride and magnesium sulfate. Nigari tofu is widely appreciated for its flavor (Tsai et al. 1981; Chang 2006). Yields increase with the concentration of coagulant up to an optimal concentration (Sun and Breene 1991). Yields with citrus and lactic acids are low, and the resulting products have an acidic taste that many people do not like (Chang 2006).

Texture of tofu varies with how quickly the curds are formed. (See *Action of the Substance*.) When added to warm soymilk, totally dissociated salts such as calcium chloride and magnesium chloride form curds quickly, and produce tofu that is coarse, granular and hard. Calcium sulfate, magnesium sulfate and GDL react slowly to produce a softer, smoother curd. Calcium sulfate reacts slowly because it is nearly insoluble in water, and few ions are available initially. GDL reacts slowly because the actual coagulant, gluconic acid, is formed slowly (Hou et al. 1997; deMan et al. 1986; Yang and James 2013).

The texture, taste, flavor, and other characteristics of the tofu vary according to bean cultivar, water/bean ratio, temperature, heating times, degree of mixing, filtration pressure, type of coagulant, coagulant concentration, and coagulation times (Beddows and Wong 1987a, b and c; Hou et al. 1997; Yang and James 2013).

Flavor of the tofu depends partly on processing. Soaking and cold grinding gives a "beany" taste. Soaking and hot grinding does not. In small scale taste tests, tofu made with calcium sulfate or nigari receive the highest ratings for flavor. Concentrations of GDL higher than 0.44% leave an acidic taste that some people find unpleasant (Hou et al. 1997; Lim et al. 1990; Lu et al. 1980; Sun and Breene 1991).

An important characteristic of tofu is its texture. Textures may be hard, soft, or silken depending on the amount of water in the tofu. Hard tofu is produced by using coagulant salts such as calcium sulfate. The curds that form are broken and added to a mold, and water is pressed out. The pressed cubes are sliced, then packed into containers and sterilized. This form of tofu is called momen tofu (Chang 2006), and it is often called "firm" tofu on packaging labels. Increasing coagulation temperatures and stirring rate after adding coagulant increases hardness (Hou et al. 1997). Hardness also increases with the amount of particulate protein in the soymilk (Cheng et al. 2005). Hard tofu has a relatively low water content (74% average). Hard tofu is used for purposes such as stir frying (Tsai et al. 1981).

Soft tofu is often used in soups. It has a higher water content (84% average) than the drier hard tofu. Soft tofu is produced by coagulants such as nigari, calcium chloride, or calcium sulfate, and by adjusting temperatures and stirring rates to produce a softer product. Water is either pressed out of the curds that form, or curds are left unpressed (Tsai et al. 1981; Chang 2006).

Silken tofu has the consistency of yogurt or custard. Curds are not pressed to remove water and whey, and the tofu has a high water concentration (87-89%). GDL can be used to make silken tofu. A preferred industrial process is to add hot soymilk and fresh GDL solution simultaneously to sterilized containers. As the GDL heats up, it is slowly converted to gluconic acid; the pH drops, and the solution slowly congeals. Silken tofu produced with GDL is produced directly in the container (deMan et al. 1986). This type of production reduces the chance of microbial contamination (Yang and James 2013). Production with GDL in this manner extends the shelf life of the tofu (Guo et al. 2005; Tsai et al. 1981).

Silken, packed tofu of this type can also be made with nigari or calcium chloride. The soymilk is cooled to 4°C and the coagulant is added. Starting at low temperatures prevents fast coagulation that would otherwise occur. Containers are filled before the solution coagulates. Then containers are heated to coagulate the tofu (Chang 2006).

GDL used alone to make hard tofu produces a rubbery product, but yields are about 20% higher than with calcium sulfate. GDL has been combined with calcium sulfate to produce hard tofu that has a smooth texture and high yield (Lu et al. 1980; Lim et al. 1990; Shen et al. 1991; Cheng et al. 2005). The addition of carrageenan to calcium sulfate or calcium acetate tofu increases yields and decreases hardness (Karim et al. 1999).

Other uses in food production

Most food uses of GDL depend on its acidic and chelating properties. GDL is used as a curing and pickling agent, a leavening agent, and as a pH control agent. When GDL is added to water, the taste is initially sweet. Then it hydrolyzes to gluconic acid, giving a slightly sweet, mildly acidic taste. In many foods, it is preferred to other acids that give stronger, more acidic tastes (Jungbunzlauer 2008; Rubico and McDaniel 1992).

Many dairy processes require a slow pH reduction. GDL can be used instead of lactic acid bacteria in production of cottage cheese, feta cheese and mozzarella (Jungbunzlauer 2008; Rankin et al. 2006). Direct acidification to produce curding is an easier process to control than production with bacterial cultures. Cheese production is also quicker. However, in the case of cottage cheese, the cultured product has a better flavor and texture (Makhal et al. 2013).

Addition of GDL to processed meat such as frankfurters controls the pH and hastens the curing and ripening process. It allows up to a 30% reduction in the amount of nitrite added, and a 75% reduction of residual nitrite (Jungbunzlauer 2008). However, when GDL and ascorbic acid were used to replace some of the nitrites in Turkish fermented sausages, the product had lower yield and poorer flavor (Yilmaz and Zorba 2010). According to 7 CFR 205.605, nitrites are not allowed in organic processed products.

GDL acidifies restructured pork and allows reduction in the amount of sodium chloride used for meat preservation (Hong et al. 2008). It is an antimicrobial, extending the shelf life of bologna and reducing total microbial counts in beef and pork products. Combined with sodium erythorbate, it helps keep myoglobin oxidized and leaves meat with a redder color (Barringer et al. 2005). Sodium erythorbate is not listed at 7 CFR 205.605 and is therefore not allowed in organic processing.

GDL is used as a leavening agent in bread. The gluconic acid produced when water is added reacts with sodium bicarbonate to produce carbon dioxide gas, causing the dough to rise. Compared to other leavening agents, it has a slow to intermediate rate of carbon dioxide release (Jungbunzlauer 2008; 2009). GDL is often added to cake mixes because it has a long shelf life, and it is used often in pastries. Up to 40% more GDL may be added than is necessary for the reaction with sodium bicarbonate. This acidifies the product and retards the growth of mold (Feldberg 1959; Jungbunzlauer 2009). Other acids used as chemical leavening agents include adipic acid, potassium tartrate, sodium acid pyrophosphate and others (Bellido et al 2009).

GDL is used to acidify and help preserve processed salad dressings. Its chelating properties help protect against oil rancidity by removing ions that catalyze oxidation of fats (PMP 2004).

It is also used as an acidulant in ready-to-eat pasta and rice. It lowers the pH and retards microbial growth. It is also used as a browning inhibitor in canned fruits and vegetables (Jungbunzlauer 2008). (See *Action of the Substance.*)

GDL is used as a chelating agent in seafood, allowing a 50-90% reduction in sulfites. In canning brine, it enhances the effects of sodium benzoate preservative by decreasing the pH (Jungbunzlauer 2008). In an organic processed product, however, sodium benzoate would not be allowed.

Non-food uses

In addition to food uses, GDL is used as a cleaning compound, a medication, and as a tobacco additive (US NIEHS 2015).

Approved Legal Uses of the Substance:

D-gluconic acid and sodium gluconate are listed in the 2004 EPA List 4A as inert ingredients exempt from tolerance (US EPA 2005).

U.S. FDA lists glucono delta-lactone as a GRAS food additive. It is approved as a curding agent for cheese (21 CFR 133.129) and as a component in canned green or wax beans (21 CFR 155.120). It is also approved as a curing and pickling agent, leavening agent, pH control agent, and sequestrant (21 CFR 184.1318).

USDA Food and Safety Inspection Service mandates that when GDL is used in restructured meat products, the product label must include GDL (9 CFR 317.8) (USDA FSIS 2008).

Action of the Substance:

GDL is used to coagulate soybean protein, producing tofu. The action of the substance in this process is complex and not completely understood (Kohyama and Nishinari 1993). A simple description is that undenatured soybean protein has the hydrophobic parts folded to the inside of protein particles. Heating denatures the protein, unfolding polypeptide chains, turning sulfhydryl groups and hydrophobic sites to the outside and leaving negative charges on the outside of the protein globules. The negative charges repel each other, keeping the denatured protein in solution (Guo and Ono 2005; Campbell et al. 2009; Kohyama et al. 1995). The addition of GDL causes more hydrogen ions to be produced, due to formation of gluconic acid. The positive ions neutralize the negative electric charges on the protein, causing coagulation through hydrophobic interactions and hydrogen bonding. Positive calcium ions also neutralize the charges, producing coagulation by the same mechanism. When coagulation occurs, a gel is formed, trapping water, lipids and other components of the soymilk (Chang 2006; Guo and Ono 2005; Campbell et al. 2009; Kohyama et al. 1995).

Soymilk contains soluble and particulate (>40 nm) proteins. Major soybean proteins include glycinin and beta-conglycin. Particulate proteins are more likely to contain glycinin, and coagulation increases with the concentration of particulates in soymilk. Coagulation and firm tofu are favored by higher concentrations of both glycinin and particulate proteins in the soymilk (Guo and Ono 2005).

GDL's function as a preservative results from the acidity produced by gluconic acid. This acidity retards the growth of many microbes. Making a substrate acidic also increases the efficacy of preservatives such as sodium benzoate, which is more effective in non-ionized form (Jungbunzlauer 2008).

GDL is used as an acidulant in ready-to-eat pasta and rice. It lowers the pH and retards microbial growth. It is also used as a browning inhibitor in canned fruits and vegetables. Browning is caused by enzymes, and lowering the pH away from the optimal pH for enzyme function slows the process. GDL also chelates ions that catalyze and accelerate the browning reaction (Jungbunzlauer 2008; PMP 2004).

In cheesemaking, the release of gluconic acid when GDL is dissolved denatures proteins, leading to the formation of curds. Other acids used in cheesemaking include lactic acid. Rennet enzymatically cleaves casein, which makes it insoluble in water and leads to curd formation. Sometimes rennet is combined with GDL or other acidulants (Rankin 2006; Lucey et al. 2000).

Microbials have a growth profile dependent on pH. GDL acidifies meat and reduces the growth of pathogens such as *Listeria* sp. In canned products, GDL makes it easier for heat to kill *Cloistridium* sp. and other pathogens (Santos and Zarzo 1995; Barringer et al. 2005). In canned goods such as asparagus, apricots, peaches, tomatoes, and other commodities, the acidity produced by GDL makes heat sterilization possible at lower temperatures. This improves the texture of the product (Heil et al. 1988; Heil et al. 1989; McGlynn et al. 1993).

In seafood such as shrimp, enzymes can cause the product to turn brown. GDL is added to chelate iron and copper ions, so that enzymes that depend on them are not able to exert their effects (Jungbunzlauer 2008; PMP 2004).

Combinations of the Substance:

GDL is generally sold in pure form to companies that produce processed food products. Additional ingredients (e.g., inert ingredients, stabilizers, preservatives, carriers, anti-caking agents, or other materials) are not generally added to commercially available forms of the substance. However, for bakery uses Jungbunzlauer sells GDL that is coated with fat to slow down carbon dioxide production (Jungbunzlauer 2009).

Food processors use the GDL they purchase in combination with other ingredients. For instance, sodium alginate, calcium carbonate and GDL are used in combination as a meat preservative (PMP 2004). Suppliers of pasta and rice use GDL in combination with sodium citrate and tricalcium phosphate to improve color and texture (PMP 2004). Bakers sometimes combine GDL with other leavening agents such as potassium acid tartrate, sodium acid pyrophosphate and monocalcium phosphate. Sodium bicarbonate is added to release carbon dioxide (PMP 2004). Tofu producers use GDL in combination with other coagulants such as magnesium chloride, nigari, and calcium sulfate (Chang 2006). GDL is used in combination with vinegar to make commercial salad dressings (PMP 2004). Jungbunzlauer combines GDL and sodium bicarbonate in a low-sodium baking powder. GDL is combined with potassium bicarbonate and corn starch in a sodium- and phosphate-free baking powder (Jungbunzlauer 2012).

Status

Historic Use:

The National Organic Standards Board (NOSB) reviewed GDL in 1995 for inclusion on the National List of Approved and Prohibited Substances as a tofu coagulant. It was not included in the Final Rule creating the National Organic Program (NOP) on December 21, 2000 (USDA Agricultural Marketing Service 2000).

A Technical Advisory Panel report written in 2002 recommended the addition of GDL to the National List as an allowed nonsynthetic for "organic" (at least 95% organic ingredients) and "made with organic" (at least 70% organic ingredients) foods (OMRI 2001). For "organic" use, reviewers suggested that the NOSB either add it with annotations, or not add it at all. For "made with organic" use, two reviewers requested annotations and a third did not. Annotations requested included "for tofu production only" and "produced by microbial fermentation" (OMRI 2002).

A Final Rule to address GDL was published on November 3, 2003 (USDA Agricultural Marketing Service 2003). According to the Final Rule, "four of the commenters requested it be added with the following annotation: 'produced through microbial fermentation of carbohydrates only.' This annotation would disallow the use of oxidation of D-glucose with enzymes, but enzymes are allowed in Sec. 205.605(a). Accordingly, this annotation is not adopted. However, the listing is amended to add the annotation 'production by the oxidation of D-glucose with bromine water is prohibited.' This will allow only the microbial and enzymes oxidation production methods."[Note: There are many chemical methods of gluconic acid synthesis other than bromine water. See Evaluation Question 1. Also, production with pure enzymes may involve Genetically Modified Organisms (GMOs).] The 2003 Final Rule added GDL to 7 CFR 205.605(a) with the following annotation: "production by the oxidation of D-glucose with bromine water is prohibited" (USDA Agricultural Marketing Service 2003).

Since then, GDL has remained on the National List at 7 CFR 205.605(a) without change throughout subsequent sunset reviews by the NOSB. At the meeting in Arlington, Virginia November 27-30, 2007, the NOSB voted 15-0 to keep GDL on the National List with the annotation intact, although some members questioned whether it was nonsynthetic (National Organic Standards Board 2007). The GDL listing and annotation were formally renewed in a Final Rule published October 9, 2008 (USDA Agricultural Marketing Service 2008).

On May 25, 2012, the NOSB voted 14-1 to keep GDL on the National List with the annotation intact. According to the Board, "review of the original recommendation, the 2002 TAP review, historical documents, the 2007 sunset recommendation, and public comments does not reveal unacceptable risks to the environment, human, or animal health as a result of the use or manufacture of this material. There is no new information contradicting the original recommendation which was the basis for the previous NOSB decisions to list and again re-list this material" (National Organic Standards Board 2012). GDL was formally renewed on the National List in a Final Rule published October 3, 2013 (USDA Agricultural Marketing Service 2013).

<u>Organic Foods Production Act, USDA Final Rule:</u>

GDL was not listed in the Organic Foods Production Act of 1990, and it was not mentioned in the Final Rule creating the NOP published October 21, 2000 (USDA Agricultural Marketing Service 2000). It is currently on the National List of Allowed and Prohibited Substances at 7 CFR 205.605(a) with the annotation, "production by the oxidation of D-glucose with bromine water is prohibited."

<u>International</u>

Canada - Canadian General Standards Board Permitted Substances List, CAN/CGSB-32.311-2006

Glucono delta-lactone is not on the Canadian General Standards Board Permitted Substances List. Specifically, it is not on the list of Non-organic Ingredients Classified as Food Additives, or Substances Permitted in Products Whose Contents are 70% or More and Less than 95% Organic Ingredients or Processing Aids (Canada 2011).

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)

Glucono delta-lactone is not mentioned in the *Codex Alimentarius* Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods. Specifically it is not listed in Annex 2 "Permitted Substances for the Production of Organic Foods." It is also not listed in Table 3 "Ingredients of Non-Agricultural Origin…" or in Table 4 "Processing Aids Which May be Use for the Preparation of Products of Agricultural Origin" (Codex 2001).

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008

Glucono delta-lactone is not mentioned in European Community Council Regulation 834/2007. However, in Article 7 "Specific Principles Applicable to the Processing of Organic Food," part b states as a principle, "the restriction of the use of food additives, of nonorganic ingredients with mainly technological and sensory functions and of micronutrients and processing aids, so that they are used to a minimum extent and only in case of essential technological need or for particular nutritional purposes" (EU ECC 2007).

Glucono delta-lactone is not mentioned in European Community Council Regulation 889/2008. Specifically, it is not mentioned in Annex 8. "Certain Products and Substances Which May be Used in Production of Processed Organic Food" (EU ECC 2008).

Japan Agricultural Standard (JAS) for Organic Production

Glucono delta-lactone is not mentioned in the Japan Agricultural Standard for Organic Production (JAS 2005).

IFOAM – Organics International (IFOAM)

Glucono delta-lactone is not listed in the IFOAM Norms for Organic Production and Processing. Specifically, it is not listed in Appendix 4, Table 1 "List of Approved Additives and Processing/Postharvest Handling Aids" (IFOAM 2012).

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Production of Glucono delta-lactone

Glucono delta-lactone (GDL) is commercially produced by crystallization from an aqueous solution of gluconic acid. Chemically, glucose is an aldohexose, which is a six-carbon sugar containing an aldehyde group. The aldehyde group can be oxidized to a carboxyl group, producing gluconic acid. When gluconic acid is dissolved in water, it reacts with itself, forming two cyclic esters called lactones: a six-carbon cyclic compound called glucono delta-lactone, and a five-carbon cyclic compound called glucono gamma-lactone. In aqueous solutions, the three compounds are in equilibrium (Ramachandran et al. 2006).

Gluconic acid, glucono delta-lactone, and glucono gamma-lactone have different solubilities in water. In concentrated aqueous solutions, glucono delta-lactone crystallizes out of solution at temperatures between 30°C and 70°C. The crystals can be isolated by filtration or centrifugation (Pasternack 1937; Rohr et al. 1983).

Production of Gluconic Acid

Production of GDL depends on a source of gluconic acid. Gluconic acid is produced by oxidation of glucose. Oxidation can be accomplished by many different processes, which can be classified as (1) oxidation by chemical methods, (2) oxidation by microbes during fermentation, or (3) oxidation by purified enzymes (Ramachandran et al. 2006; Rohr et al. 1983; Anastassiadis and Morgunov 2007).

(1) Oxidation by Chemical Methods

Early production involved chemical oxidation with bromine water or bleach, but purification was difficult and labor-intensive. A modification of the process was electrolysis of a glucose solution containing bromide ion (Isbell et al. 1932). Catalytic oxidation with dissolved oxygen and platinum, palladium and other catalysts has been used. Many of these processes have drawbacks that make them uneconomical (de Wilt 1972). Oxidation by bromine water is specifically prohibited in the annotation for GDL at 7 CFR 205.605(a).

(2) Oxidation by Microbes during Fermentation

Most of the industrial production of gluconic acid is currently accomplished by microbial fermentation. Fungi such as *Aspergillus niger*, *Penicillium* spp., and others produce a glucose oxidase enzyme (*beta*-D-glucose: oxygen 1-oxidoreductase, E.C. 1.1.3.4) that oxidizes glucose to gluconic acid. In fact, glucose oxidase was first discovered in 1928 in an extract of *Aspergillus niger*. The fermentations are mostly accomplished by a submerged culture process wherein glucose and growth media are sterilized, and then the microbe is added to a vigorously stirred and oxygenated reaction vessel (Anastassiadis and Morgunov 2007; Wong et al. 2008; May et al. 1934). In a batch process, reactants are added initially and the reaction is allowed to run to completion. In a fed batch process, the glucose is added at convenient intervals to increase the concentration of product in the batch. In a continuous process, the reaction products are continuously siphoned off and new reactants are continuously added until the microbe loses potency.

The bulk of the production today is with submerged culture using *Aspergillus niger* as the active microbial (Blom et al. 1952; Anastassiadis and Morgunov 2007). To produce gluconic acid, the fermentation has to be done near pH 6, and the reaction product is a gluconic acid salt (Blom et al. 1952). The *Aspergillus niger* submerged culture fermentation is discussed in more detail below.

Some production by continuous submerged culture process has involved the use of the yeast-like organism, *Aureobasidium pullulans*. Best yields are at pH 6.5. At this pH, gluconic acid is mostly present as the gluconate salt. Other pH values led to poor product selectivity and yields (Seiskari et al. 1985; Anastassiadis et al. 2005; Ramachandran et al. 2006).

Gluconic acid can also be produced by fermentation with *Aspergillus niger* immobilized in gels or on various supports, such as non-woven polypropylene. The process is slow and the product is contaminated with citric acid, making cleanup and isolation more difficult. Bacteria such as *Gluconobacter oxydans* have also been used as the active microbials. The advantage is that the bacterial dehydrogenase enzyme (GDH, E.C. 1.1.99.17) is not affected by low pH, so that gluconic acid can be isolated directly. The disadvantage is that the bacterial enzymes can oxidize gluconic acid further to keto acids. This oxidation makes purification of gluconic acid more difficult (Stubbs et al. 1940; Seiskari et al. 1985; Ramachandran et al. 2006).

(3) Oxidation by Purified Enzymes
In the 1960s, some gluconic acid was produced with immobilized, purified glucose oxidase and catalase enzymes. Due to the current high cost of the purified enzymes, that method is seldom used today (Pazur and Kleppe 1964; Ramachandran et al. 2006; Anastassiadis and Morgunov 2007). Also, commercial enzymes today are likely to originate from Genetically Modified Organisms (GMOs), since GMO microbials can be engineered to overexpress the enzyme needed (Bankar et al. 2009; Singh and Kumar 2007; Schuster et al. 2002).

Submerged Fermentation with *Aspergillus niger*
In concentrated glucose solutions at optimal pH 5 and in an excess of oxygen, glucose oxidase from *Aspergillus niger* oxidizes glucose to glucono delta-lactone, which then hydrolyzes in the acidic solution to gluconic acid (Rohr et al. 1983).

The active oxidation center in glucose oxidase is a Flavin Adenine Dinucleotide (FAD) component. When glucose is oxidized, FAD of the enzyme is reduced. The reduced FAD is then oxidized by reaction with oxygen, forming hydrogen peroxide. Hydrogen peroxide is converted to water and oxygen by the *Aspergillus niger* enzyme catalase, completing the reaction cycle (Ramachandran et al. 2006).

As the fermentation proceeds, the solution becomes more acidic due to production of gluconic acid. When the pH drops below 3.5, glucose oxidase is inactivated and the fermentation mechanism shifts toward production of citric acid. In fact, production of citric acid was one of the first industrial uses of *Aspergillus niger*. For production of gluconic acid, alkaline materials have to be added to the fermentation to neutralize the gluconic acid produced so that the process can continue to completion (Rohr et al. 1983; Soccol et al. 2006).

Most submerged *Aspergillus niger* fermentations today use various modifications of the Blom process to produce a gluconic acid salt (Blom et al. 1952; Ramachandran et al. 2006). In the Blom process, a growth medium containing fermentation nutrients and glucose feedstock is sterilized by heat. The growth medium contains glucose, corn steep liquor and urea, along with nutrient salts of magnesium sulfate, potassium dihydrogen sulfate, and ammonium phosphate. Initial pH is adjusted to 6.0-6.5. An *Aspergillus niger* inoculant solution is then added. Sodium hydroxide is added on a regular basis to maintain pH above 5. Sometimes calcium carbonate is used instead of sodium hydroxide. Fermentation times are about 10 to 45 hours at 30°C.

The glucose source used by Blom et al. came from corn starch. Currently, most production uses pure glucose monohydrate or glucose syrup. Other substrates include sucrose, hydrolysed starch, hydrolysed corn starch (hydrol), cane molasses, whey, waste paper, grape must, and other materials (Matsui et al. 2013; Mafra et al. 2015; Ramachandran et al. 2006).

The *Aspergillus niger* strain used by Blom et al. was NRRL-3. [NRRL-3 is also called CBS 120.49 and ATCC 1015.] The *Aspergillus niger* organism is discussed further in Evaluation Question 2.

Fermentation proceeds with high aeration and vigorous mixing. Since the gluconic acid produced tends to inactivate glucose oxidase, sodium hydroxide is continuously added. The substance produced by fermentation is actually sodium gluconate. Sodium gluconate yields of >90% were produced by this method (Blom et al. 1952; Rohr et al. 1983). Later refinements have led to almost quantitative conversion from glucose (>98%) (Ramachandran et al. 2006).

To isolate sodium gluconate, the fungus is filtered off and the solution is concentrated then dried in a drum dryer, producing solid sodium gluconate. This is dissolved in water and recrystallized to give a product >99% pure. The method's simplicity and reliability has made it the industrial favorite. However, production of gluconic acid involves another step, such as running the solution through an ion exchange column (Blom et al. 1952; Rohr et al. 1983).

Most commercial gluconic acid production today proceeds through sodium gluconate or calcium gluconate in modifications of the Blom et al. 1952 process, and most GDL used in organic processing, likely comes through this pathway (Ramachandran et al. 2006; Anastassiadis and Morgunov 2007).

Suppliers of GDL include Jungbunzlauer, Roquette, PMP, Omicron, Purac, Westco and others. Gluconic acid and gluconates are supplied by Pfizer, Bristol-Meyers, Premier Malt Products, Roquette, Benckiser, and Fujisawa. Annual world production of gluconic acid is 60,000 to 100,000 metric tons (Singh and Kumar 2007; Xavier et al. 2010).

<u>Evaluation Question #2:</u> Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)). Discuss whether the petitioned substance is derived from an agricultural source.

GDL produced by fermentation with *Aspergillus niger* is currently classified as nonsynthetic as indicated by its listing under (a), *Nonsynthetics allowed*, of 7 CFR 205.605.

Production of GDL by dissolving gluconic acid in water followed by fractional crystallization is a nonsynthetic process (Ramachandran et al 2006). Dissolving gluconic acid in water does not involve a chemical reaction. Spontaneous reaction of gluconic acid in water to form glucono delta-lactone (GDL) is a natural, nonsynthetic process that does not involve addition of chemical reagents. GDL formed from gluconic acid occurs naturally in many foods. Crystallization from water is a physical process that does not involve a synthetic chemical reaction.

The gluconic acid needed to produce GDL is created by naturally occurring biological processes. The major production pathway is oxidation of glucose by aqueous submerged cultures of *Aspergillus niger* (Ramachandran et al. 2006; Blom et al. 1952) as described in Evaluation Question 1. More specifically, production of gluconic acid by *Aspergillus niger* in the Blom process proceeds through five distinct steps: (1) initial synthesis of glucono delta-lactone and gluconic acid by fermentation, (2) addition of sodium hydroxide or calcium carbonate to produce a gluconate salt, (3) isolation of sodium or calcium gluconate, (4) recrystallization of the gluconate salt, and (5) conversion of gluconate salts to gluconic acid. The chemical and biological processes are described below for each step.

(1) Initial Synthesis

Oxidation of glucose to glucono delta-lactone by *Aspergillus niger* is a nonsynthetic spontaneous enzymatic process. Oxidation by glucose oxidase also occurs naturally, and glucono delta-lactone is present in food. Glucono delta-lactone then hydrolyzes to gluconic acid in a spontaneous process that is also nonsynthetic. Hydrolysis occurs naturally whenever glucono delta-lactone is added to water (Ramachandran et al. 2006).

(2) Addition of Alkali, Producing a Salt

When sodium hydroxide is added to the fermentation, an acid-base reaction occurs, forming sodium gluconate.

(3) Isolation of the Salt

Isolation of sodium gluconate solid by evaporation of the fermentation medium is a nonsynthetic process. Nothing new is formed, and the sodium ions and gluconate ions in solution combine to form a solid salt when the water is removed. No chemical reaction has occurred. The ions present in solution are now in a solid matrix.

(4) Recrystallization of the Salt
The solid sodium gluconate is dissolved in water and recrystallized. At this point most impurities such as sodium hydroxide, growth media salts, and other substances introduced during fermentation are removed. The sodium gluconate salt is unchanged by recrystallization, and the purity is >99%.

(5) Conversion of Salt to Gluconic Acid
When the solid sodium gluconate is dissolved in water and passed through an ion exchange column, the polymeric beads in the column act like a filter, removing sodium ions. Sodium ions are electrostatically attached to the beads. This is a form of ionic attraction, and not a chemical reaction where bonds are broken and new bonds are formed.

Hydrogen ions from the column are then exchanged for the sodium ions. The hydrogen ions react with gluconate ions, forming gluconic acid. The calcium gluconate produced by glucose fermentation by *Aspergillus niger* in the presence of calcium carbonate can also be converted into gluconic acid. Either an ion exchange column is used, or sulfuric acid is added to a calcium gluconate solution. Insoluble calcium sulfate forms, which is filtered off to leave a solution of gluconic acid. This is an acid-base reaction where hydrogen ions from sulfuric acid react with gluconate ions, forming gluconic acid.

Aspergillus niger
Aspergillus niger is often found in nature. It grows on a wide variety of substrates, producing spores. The initial fermentation experiments in the 1930s used strains isolated from the environment. Some of these were archived by American Type Culture Collection (ATCC) and other organizations. As the process became more industrialized, special production strains were produced by mutation (Soccol et al. 2006). The strain NRRL-3 (also called CBS 120.49, ATCC 1015) used by Blom et al. (1952) is a wild type strain. It is one of the most commonly used industrial strains (Frisvad et al. 2011).

Up to the 1980s, special strains used for industrial fermentation came from mutation with chemicals, UV light, and gamma radiation. As genetic engineering techniques became more ubiquitous, some GMO strains were created. Many of the GMO strains of *Aspergillus niger* are used to produce purified enzymes that have industrial importance (Soccol et al. 2006; van Dijck et al. 2003).

Mutants and GMOs are seldom used in gluconic acid production from pure glucose because the wild industrial strains available can produce nearly quantitative conversions in 24 to 48 hours (Ruijter et al. 2002). However, there are instances where mutants and GMOs are used, especially for complex substrates (Purane et al. 2012; Singh and Kumar 2007).

FDA requirements for GRAS use of GDL as a food additive stipulate that microbes used for production be "non-pathogenic and non-toxicogenic to man and other animals" (21 CFR 184.1318). The safety of *Aspergillus niger* is discussed in Evaluation Question 10.

Similarities to Citric Acid Production
Citric acid is produced by a similar process involving *Aspergillus niger* fermentation of molasses, sucrose, starch and other substrates. In that process, mutant strains of *Aspergillus niger* are used. The product is isolated by the addition of lime to produce the insoluble salt, tricalcium citrate tetrahydrate. The salt is treated with sulfuric acid, producing soluble citric acid and insoluble calcium sulfate. The calcium sulfate is filtered off and the solution is concentrated until citric acid crystallizes. Alternatively, removal of the citric acid from the fermentation medium is carried out by solvent extraction or by more esoteric methods (Soccol et al. 2006; OMRI 2015). Citric acid produced by this process is listed as nonsynthetic at 7 CFR 205.605(a). However, the salts produced by the reaction of citric acid with bases such as sodium hydroxide are listed as synthetic at 7 CFR 205.605(b).

Agricultural Sources

GDL is classified as a nonagricultural substance as indicated by its listing at 7 CFR 205.605, *Nonagricultural (nonorganic) substances*. The starting materials, such as cornstarch or molasses that are necessary for production of gluconic acid are agricultural products.

Evaluation Question #3: If the substance is a synthetic substance, provide a list of nonsynthetic or natural source(s) of the petitioned substance (7 CFR § 205.600 (b) (1)).

GDL produced by fermentation with *Aspergillus niger* is currently classified as nonsynthetic as indicated by its listing under (a), *Nonsynthetics allowed*, of 7 CFR 205.605. There are no other convenient nonsynthetic sources. Fermentation with other organisms is less convenient, and involves cleanup and isolation steps that are possibly synthetic. Production by purified enzymes is expensive, and may involve GMOs (see Evaluation Question 1).

Evaluation Question #4: Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices (7 CFR § 205.600 (b)(5)). If not categorized as GRAS, describe the regulatory status.

FDA lists glucono delta-lactone as a GRAS food additive. It is used as a curding agent for cheese, a coagulant for tofu (21 CFR 133.129), and as a component in canned green or wax beans (21 CFR 155.120). It is a GRAS food additive as a curing and pickling agent, leavening agent, pH control agent, and sequestrant (21 CFR 184.1318).

Evaluation Question #5: Describe whether the primary technical function or purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR § 205.600 (b)(4)).

GDL can be used to help preserve food, but it is also used in making tofu, as a leavening agent in bread, and in cheesemaking (FASEB 1981; PMP 2004). It is used to make preservatives more effective, reducing the amount of salt needed to preserve meat, and reducing the amount of nitrites needed in sausage (Jungbunzlauer 2008; PMP 2004).

Its function as a preservative depends partly on the acidity produced by gluconic acid. This acidity retards growth of some microbes. Making a substrate acidic also increases efficacy of preservatives such as sodium benzoate, which is more effective in non-ionized form. However, use of sodium benzoate would not be allowed in organic processing. GDL also helps preserve food by chelating metal ions that catalyze decay (Jungbunzlauer 2008). The mechanism of preservation is discussed further in *Action of the Substance*.

Evaluation Question #6: Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR § 205.600 (b)(4)).

GDL is not used primarily to improve flavors, colors, textures, or nutritive values lost in processing. It is used as a coagulant, a leavening agent, a sequestrant, and for other uses. When used in canning (21 CFR 155.120), it can prevent the loss of color and texture from excessive heat processing. When used in frankfurters, it accelerates curing and production of color (Jungbunzlauer 2008; Heil et al. 1988; Acton and Dick 1977).

<u>Evaluation Question #7</u>: Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR § 205.600 (b)(3)).

Addition of GDL is nutritionally equivalent to addition of glucose (UNEP 2006; Stetten and Stetten 1950). Addition of GDL increases the number of calories available. The physical structure of proteins are changed when GDL is used as a coagulant for tofu and cottage cheese, but nutrition is not affected (Chang 2006).

Addition of GDL in quantities greater than 0.44% by weight (25 mM) to soybean milk gives a sour taste to the resulting tofu (Tsai et al. 1981).

The use of GDL instead of calcium salts for tofu coagulation results in a tofu that has less calcium and phosphate ion, and thus less nutrition. GDL tofu also has a higher water content (90% versus 84-88% for calcium ion coagulants) (Tseng et al. 1977).

When used in bread, the gluconic acid produced reacts with baking soda, releasing carbon dioxide gas and causing the dough to rise. Nutrition is generally not affected, but taste of the final product depends on the leavening agent used (Lai and Lin 2006). GDL is also used in sodium free baking powder. In this instance, the nutrition is changed because potassium bicarbonate is substituted for sodium bicarbonate (Jungbunzlauer 2012).

<u>Evaluation Question #8:</u> List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR § 205.600 (b)(5)).

Standard of purity for GDL is listed in the *Food Chemicals Codex*. It must be >99% pure. It can contain up to 4mg/kg of lead. Standards for other heavy metals are not listed. Up to 0.5% glucose contaminant is allowed (Food Chemicals Codex 2004). No reports of GDL contamination were found on the Internet or in CAB or Pub Med databases as of September 29, 2015.

<u>Evaluation Question #9:</u> Discuss and summarize findings on whether the manufacture and use of the petitioned substance may be harmful to the environment or biodiversity (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

Manufacture of GDL by fermentation is not expected to be harmful to the environment or biodiversity. All manufacturing steps of gluconic acid and GDL take place in closed systems in all countries of production. Only maintenance workers and laboratory personnel are potentially exposed to any components (UNEP 2006). The fermentation medium contains nutrient salts, glucose, and *Aspergillus niger* (See Evaluation Question 1).

Aspergillus niger is a ubiquitous fungus that occurs naturally, so it is already present in the environment. It is generally thought to be safe, but human infections have occurred in cases of compromised immune systems (Schuster et al. 2002; UNEP 2006) (see Evaluation Question 10). With batch fermentation, fungi are recycled through many batches. Solid microbial residues can be composted (Blom et al. 1952).

Fermentation media contains nutrient salts of low toxicity. Magnesium sulfate, dihydrogen phosphate and ammonium phosphate are used. Amounts not used for microbial growth could be recovered during recrystallization of gluconate salts (Blom et al. 1952).

<u>Evaluation Question #10:</u> Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

The U.S. FDA classifies GDL as a permitted food additive that is GRAS (Generally Recognized as Safe) (21 CFR 184.1318). The Pub Med Database contains no reported effects of GDL on human health (Pub Med, October 5, 2015).

To determine possible human health effects, the toxicity of GDL should be considered. GDL is metabolically related to glucose, gluconic acid and gluconate salts. In the body, about 10% of all glucose ingested is converted to gluconate. The gluconate is then either phosphorylated and oxidized further, or is converted back to glucose. This means that there is a natural exposure to gluconate from the food supply. On average, about 275 g of glucose are ingested daily, resulting in a gluconate exposure of about 450 mg/kg/day for a 60 kg human (UNEP 2006).

GDL is mostly metabolized in the same way as glucose and is nutritionally equivalent (Stetten and Stetten 1950; Stetten and Topper 1953; UNEP 2006).

Gluconates have low toxicity. The oral LD50 of sodium gluconate in a rat is >2000 mg/kg. The oral LD50 of potassium gluconate in a rat is 6060 mg/kg, and the oral LD50 of GDL in a rat is 5940 mg/kg (JECFA 1999; UNEP 2006). [In toxicology, the LD50 refers to the amount of a substance that will kill half the test population.]

GDL is not mutagenic, and does not cause birth defects or reproductive problems. Rats fed 10% GDL in rat feed for 24 months (4920-5720 mg/kg) showed no adverse effects (UNEP 2006).

When 10 g doses of GDL were fed to humans, about 7-15% was recovered from urine and the rest was metabolized. Doses of 5 g were completely metabolized. Doses of 15 g administered frequently caused diarrhea (FASEB 1981; JECFA 1999).

GDL Consumption

In the U.S. in 1970, annual consumption was 73,000 kg and the daily per capita average was 0.9 mg GDL. Amounts added to food (expressed as weighted mean) were: 0.31% baked goods, 1.01% milk products, 1% cheese, 0.25% drinks, and 0.15% meat (FASEB 1981).

Current rates of addition are limited to 0.5% (5 g/kg) in sausages, except 1% (10g/kg) is allowed for Genoa sausage. About 0.3% is added to restructured meat, and 0.5% is added to pork liver pate (PMP 2004).

About 0.4% to 1.2% is added to pasta noodles. In bakery products, 1 gram is added for every 0.472 g of baking soda. This is about 4% of the total weight of flour in cake mixes, bread, and pastries (Feldberg 1959). About 1% is added to canned fruit and vegetables. Amount of GDL added to cheese is 12% of milk solids. In tofu, GDL is 0.3% or 0.4% of the weight of soymilk (PMP 2004).

The annual worldwide consumption of gluconic acid is about 87,000 tons, or 87 million kg (191 million lbs). Of this, about 30,000 tons or 30 million kg (66 million lbs) are used in food. From this data, one can make a very rough estimate of GDL per capita consumption. Assuming all food uses are GDL, and it is consumed by a billion people in industrialized countries, per capita consumption would be 30 grams a year, or 82 mg/day. This is a high estimate because gluconate salts as well as GDL are used in food (Singh and Kumar 2007).

Another estimate of worldwide annual consumption of GDL is 10,000 to 20,000 tons (UNEP 2006). This would give a very rough estimate of per capita consumption in industrialized countries of 10-20 g/year or 27-55 mg/day.

Per capita GDL use has increased since 1970 from 0.9 mg to at least 27 mg/day, but amounts used are well below any toxic thresholds (FASEB 1981; UNEP 2006; JECFA 1999). [Note: This is a very rough estimate. The 1970 figure refers to the U.S., and the current estimate is based on worldwide consumption.]

Health Effects of *Aspergillus niger*

U.S. FDA requirements for GRAS use of GDL as a food additive stipulate that microbes used for production be "non-pathogenic and non-toxicogenic to man and other animals" (21 CFR 184.1318). *Aspergillus niger* is thought to be a safe microbe. It occurs naturally on many food plants. Humans are exposed to spores every day in gardens and other outdoor situations without harm. However, infections of humans can occur when immune systems are compromised. Some people may be allergic to the spores. Toxic materials produced in some cases include nigracillin, plant malformins, and napthto-gamma-pyrones (Schuster et al. 2002).

A. niger does not produce aflatoxins or trichothenes, but one review found about 6% of *A. niger* isolates produce ochratoxin A that can damage kidneys (Schuster et al. 2002). Later work found the frequency of occurrence was higher, especially in industrial strains. Toxins such as fumonisins can also be produced (Frisvad et al. 2011).

A. niger has been well studied, and three strains: NRRL-3, CBS 513.88 and ATCC 1015 have had their genomes sequenced (Baker 2006). Most strains have the genes to produce fumonisins and ochratoxins. Agar is the most favorable growth medium for production of the toxins. In one experiment, 83% of industrial strains grown on agar produced fumonisins, 33% produced ochratoxins, and 26% produced both (Frisvad et al. 2011).

Therefore, many industrial strains will produce toxins when grown on agar. But if any toxins are produced during industrial production, amounts produced are diluted below the level of detection. According to Frisvad et al. (2011), "numerous batches of all products have been tested for cytotoxicity, carcinogenicity and other tests for product approval. These are all tests which clearly would have picked up significant concentrations of ochratoxins and/or fumonisins, indicating that these toxins may not be produced under industrial submerged growth conditions" (Frisvad et al. 2011).

Evaluation Question #11: Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

The original petitioned use for GDL was for production of tofu, especially silken tofu. Silken tofu can be produced with coagulants other than GDL, but the process is not as convenient, because the soymilk must be chilled to slow coagulation. However, the flavor may be better (Chang 2006).

GDL is added to foods to replace older practices such as making bread with yeast or culturing cheese with bacteria. Yeast is still used extensively to make bread. As it grows, it produces carbon dioxide that causes the bread to rise. Organic acids are produced as well as carbon dioxide, and these give yeast bread its unique flavor. Making bread this way is time consuming, and the bread must be kneaded (Lai and Lin 2006).

Bread can also be made to rise by reaction between sodium bicarbonate and acidic chemical leaveners. The process is more convenient than the use of yeast. GDL is especially convenient for frozen or refrigerated dough because cold temperatures adversely affect baking yeast. GDL is slowly converted to gluconic acid as the dough is heated, causing production of carbon dioxide. Other chemical leaveners such as potassium tartrate can also be used. Each acidic chemical leavener has a different reaction profile as the temperature increases (Lai and Lin 2006; PMP 2004).

Bacterial cultures can be used in cheesemaking instead of acidification with GDL. The taste of the cultured cheese is better, but use of GDL is easier and produces a more uniform product (Rankin et al. 2006). Other chelating agents such as ethylenediaminetetraacetic acid (EDTA) can be used to replace GDL's chelating actions. Sulfites can be used to preserve seafood, and nitrites can be used to preserve sausages (PMP 2004). However, EDTA, sulfites and nitrites would not be allowed in organic processed products.

One advantage of GDL is that it can help replace materials that are more toxic, such as nitrites in sausage, but some believe use of GDL in sausage leads to inferior taste (Toldra 2006). GDL is used because of convenience, economics, to increase yields, and for long shelf life. There are alternatives that in some cases produce a better tasting product. In other cases, taste suffers when GDL is replaced (PMP 2004; Toldra 2006; Rankin 2006; Lai and Lin 2006; Chang 2006).

Evaluation Question #12: Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).

Organic silken tofu made with calcium sulfate and nigari and without GDL is commercially available (e.g. Nasoya Organic Silken Tofu). GDL does not appear to be necessary to make silken tofu (Nasoya 2015; Chang 2006).

Yeast can be used to make bread instead of using chemical leaveners such as GDL (Lai and Lin 2006). Lactic acid bacteria and rennet can be used in cheesemaking to coagulate milk (Rankin 2006).

GDL can be replaced by other acids in canning processes, but other acids may produce less flavorful food (PMP 1994; McGlynn et al. 1993; Rubico and McDaniel 1992). GDL has a bland taste that does not intrude on the quality of the product.

Evaluation Information #13: Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR § 205.600 (b) (1)).

Juice from organic lemons could be used as a coagulant for tofu (Sanjay et al. 2008). However, the lemon juice may give an undesirable flavor, and tofu produced with GDL and other coagulants may have a better taste (Chang 2006). Although the Internet features a number of recipes for tofu coagulated with lemon juice, no commercial organic products are currently available.

References

Acton, J.C. and R.L. Dick. "Cured Pigment and Color Development in Fermented Sausage Containing Glucono-Delta-Lactone." *J. Food Protection* 40, no. 6 (1977): 398-401.

Anastassiadis, S. and I.G. Morgunov. "Gluconic Acid Production." *Recent Patents on Biotechnology* 1, no. 2 (2007): 1-14.

Anastassiadis, S., A. Aivasidis, C. Wandrey et al. "Process Optimization of Continuous Gluconic Acid Fermentation by Isolated Yeast-Like Strains of *Aureobasidium pullulans*." *Biotechnol. Bioeng*. 91, no. 4 (2005): 494-501.

Baker, S.E. "*Aspergillus niger* Genomics: Past, Present and into the Future." *Med. Mycol*. 44 (2006); S17-S21.

Bankar, S.B., M.V. Bule, R.S. Singhal et al. "Glucose Oxidase—an Overview." *Biotechnol. Advances* 27 (2009): 489-501.

Barringer, S.A., J. A. Ali, H.J. Chung. "Electrostatic Powder Coating of Sodium Erythorbate and GDL to Improve Color and Decrease Microbial Counts on Meat." *Inn. Food Sci. Emerg. Technol*. 6 (2005): 189-193.

Beddows, C.G. and J. Wong. "Optimization of Yield and Properties of Silken Tofu from Soybeans. I. The Water-Bean Ratio." *Intl. J. Food Sci. Technol*. 22 (1987a): 15-21.

Beddows, C.G. and J. Wong. "Optimization of Yield and Properties of Silken Tofu from Soybeans. II. Heat Processing." *Intl. J. Food Sci. Technol*. 22 (1987b): 23-27.

Beddows, C.G. and J. Wong. "Optimization of Yield and Properties of Silken Tofu from Soybeans. III. Coagulant Concentration, Mixing, and Filtration Pressure." *Intl. J. Food Sci. Technol*. (1987c): 29-34.

Bellido, G.G., M.G. Scanlon, J.H. Page. "Measurement of Dough Specific Volume in Chemically Leavened Dough Systems." *J. Cereal Sci*. 49 (2009): 212-218.

Blom, R.H., V.F. Pfeifer, A.J. Moyer et al. "Sodium Gluconate Production: Fermentation with *Aspergillus niger*." *Industrial and Engineering Chemistry* 44, no. 2 (1952): 435-440.

Campbell, L.J., X. Gu, S.J. Dewar et al. "Effects of Heat Treatment and Glucono-Delta-Lactone-Induced Acidification on Characteristics of Soy Protein Isolate. *Food Hydrocolloids* 23 (2009): 344-351.

Canada. 2011. *Organic Production Systems Permitted Substances Lists (CAN/CGSB-32-311-2006).* Canadian General Standards Board, Ottawa, Ontario, Canada. 24 pp.

Chang, K.C. 2006. "Chemistry and Technology of Tofu Making." In: Y.H. Hui ed. *Handbook of Food Science, Technology, and Engineering*, Volume 4 (Boca Raton: CRC/Taylor and Francis), pp. 171-1 to 171-24.

Cheng, Y., N. Shimizu and T. Kimura. "The Viscoelastic Properties of Soybean Curd (Tofu) as Affected by Soymilk Concentration and Type of Coagulant." *Intl. J. Food Sci. Technol*. 40 (2005): 385-390.

Codex Alimentarius. 2001. *Codex Alimentarius: Organically Produced Foods*. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Rome, Italy. 73 pp. ftp://ftp.fao.org/docrep/fao/005/Y2772e/Y2772e.pdf

deMan, J.M., L. deMan and S. Gupta. "Texture and Microstructure of Soybean Curd (Tofu) as Affected by Different Coagulants." *Food Structure* 5, no. 1 (1986): 83-89.

de Wilt, H.G.J., "Part I. Oxidation of Glucose to Gluconic Acid." *Ind. Eng. Chem. Prod. Res. Develop*. 11, no. 4 (1972): 370-373.

EU ECC. 2007. Council Regulation (EC) No. 834/2007 of 28 June 2007." *Official Journal of the European Union* 189 (2007): 1-22. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:189:0001:0023:EN:PDF

EU ECC. 2008. Commission Regulation (EC) No. 889/2008 of 5 September 2008. *Official Journal of the European Union* 250 (2008): 1-84.

FASEB (Federation of American Societies for Experimental Biology). 1981. "Evaluation of the Health Aspects of Glucono Delta-Lactone as a Food Ingredient." Bethesda, MD. 18 pp. https://www.faseb.org/Portals/2/PDFs/LSRO_Legacy_Reports/1981_SCOGS%20II-11%20Glucono%20Delta-Lactone.pdf

Feldberg, C. "The Use of Glucono Delta-Lactone in Bakery Products." *The Bakers Digest* October (1959):46-49.

Frisvad, J.C., T.O. Larsen, U. Thrane et al. "Fumonisin and Ochratoxin Production in Industrial *Aspergillus niger* Strains." *PLoS ONE* 6, no. 8 (2011): e23496

Food Chemicals Codex (National Institute of Medicine, Committee on Food Chemicals Codex). *Food Chemicals Codex*, 5th ed., 2004. The National Academies Press, Washington, DC. p. 194 of 998 pp.

Guo, S.T. and T. Ono. "The Role of Composition and Content of Protein Particles in Soymilk on Tofu Curdling by Glucono Delta Lactone or Calcium Sulfate." *Food Chem. Toxicol*. 70, no. 4 (2005): C258-C262.

Heil, J.R., M.J. McCarthy and R.L. Merson. "Influence of Gluconic Acid on Enzyme Inactivation and Color Retention in Canned Apricots and Peaches." *J. Food Sci*. 53, no. 6 (1988): 1717-1719.

Heil, J.R., M.J. McCarthy, D.J. McIntyre et al. "Influence of Gluconic Acid on Thermal Processing Requirements for Canned Whole Peeled Tomatoes." *J. Food Processing and Preservation* 13 (1989): 431-445.

Hong, G.P., S.H. Ko, M.J. Choi et al. "Effect of Glucono Delta Lactone and kappa-Carrageenan Combined with High Pressure Treatment on the Physico-chemical Properties of Restructured Pork." *Meat Sci*. 79 (2008): 236-243.

Hou, H.J., K.C. Chang, and M.C. Shih. "Yield and Properties of Soft Tofu as Affected by Coagulation Method." *J. Food Sci*. 62, no. 4 (1997): 824-827.

IFOAM (International Federation of Organic Agriculture Movements). 2012. *IFOAM Norms for Organic Production and Processing*. 32 pp.

Isbell, H.S., H.L. Frush and F.J. Bates. "Manufacture of Calcium Gluconate by Electrolytic Oxidation of Dextrose." *Ind. Eng. Chem*. 24, no. 4 (1932): 375-378.

JAS (Japanese Agricultural Standard). 2005. Japanese Agricultural Standard of Organic Agricultural Products. 8 pp.

JECFA (Joint FAO/WHO Expert Committee on Food Additives, International Programme on Chemical Safety, World Health Organization). "Safety Evaluation of Certain Food Additives: Glucono Delta Lactone and the Calcium, Magnesium, Potassium and Sodium Salts of Gluconic Acid." *WHO Food Additive Series* 42 (1999): 203-212. http://www.inchem.org/documents/jecfa/jecmono/v042je12.htm

Jungbunzlauer. 2008. "Glucono Delta-Lactone: Uniqueness in Acidification." Jungbunzlauer Factsheet, Jungbunzlauer, Basel, Switzerland. 5 pp.

Jungbunzlauer. 2009. "Glucono Delta-Lactone (GDL): a Natural Way of Leavening." Jungbunzlauer Factsheet. Jungbunzlauer, Basel, Switzerland. 9 pp.

Jungbunzlauer. 2012. "Sodium Free Baking Powder Based on Glucono Delta Lactone." Reprint from Wellness Food Europe, no. 3. Jungbunzlauer, Basel, Switzerland. 6 pp.

Karim, A.A., G.A. Sulebele, M.E. Azhar et al. "Effect of Carrageenan on Yield and Properties of Tofu." *Food Chem*. 66 (1999): 159-165.

Kohyama, K. and K. Nishinari. "Rheological Studies on the Gelation Process of Soybean 7S and 11S Proteins in the Presence of Glucono Delta-Lactone." *J. Agric. Food Chem*. 41 (1993): 8-14.

Kohyama, K., Y. Sano and E. Doi. "Rheological Characteristics and Gelation Mechanism of Tofu (Soybean Curd*). J. Agric. Food Chem*. 43 (1995): 1808-1812.

Lai, H.M. and T.C. Lin. 2006. "Bakery Products." In: Y.H. Hui ed. *Handbook of Food Science, Technology, and Engineerin*g, Volume 4 (Boca Raton: CRC/Taylor and Francis), pp. 148-1 to 148-31.

Lu, J.Y., E. Carter and R.A. Chang. "Use of Calcium Salts for Soybean Curd Preparation." *J. Food Sci*. 45 (1980):32-34.

Lim, B.T., J.M. deMan, L. deMan et al. "Yield and Quality of Tofu as Affected by Soybean and Soymilk Characteristics. Calcium Sulfate Coagulant." *J. Food Sci*. 55, no. 4 (1990): 1088-1107.

Lucey, J.A., M. Tamehana, H. Singh et al. "Rheological Properties of Milk Gels Formed by a Combination of Rennet and Glucono Delta-Lactone.*" J. Dairy Res*. 67 (2000): 415-427.

Mafra, A.C.O., F.F.Furlan, A.C. Badino et al. "Gluconic Acid Production from Sucrose in an Airlift Reactor using a Multi-Enzyme System." *Bioprocess. Biosys. Eng*. 38 (2015): 671-680.

Makhal, S., S.K. Kanawjia and A. Giri. "A Dual Acidification Process for the Manufacture of Direct Acidified Cottage Cheese." *Intl. J. Dairy Technol*. 66, no. 4 (2013): 552-560.

Matsui, T., K. Tooyama and S. Sato. "Simultaneous Saccharification of Corn Starch in Gluconic Acid Production by *Aspergillus niger* Immobilized on Nonwoven Fabric in a Pressurized Reactor." *J. Microb. Biochem. Technol.* 5, no. 4 (2013): 88-91.

May, O.E., H.T. Herrick, A.J. Moyer et al. "Gluconic Acid: Production by Submerged Mold Growths under Increased Air Pressure." *Ind. Eng. Chem*. 26, no. 5 (1934): 575-578.

McGlynn, W.G., D.R. Davis and F. Honarmand. "Gluconic Acid Influences Texture and Color of Canned Asparagus." *J. Food Sci*. 58, no. 3 (1993): 615.

Nasoya. 2015. "Vita Soy Organic Silken Tofu Does Not Use GDL." Personal communication, Irene Taylor. Vita Soy USA, One New England Way, Ayer, MA 01432. http://www.nasoya.com/products/organic-non-gmo-tofu/silken-tofu

National Organic Standards Board. 2007. "Formal Recommendation: Glucono Delta Lactone." Washington DC: USDA-AMS-NOP. November 30, 2007. 3pp. http://www.ams.usda.gov/sites/default/files/media/NOP%20Final%20Sunset%20Rec%20Glucono%20Delta%20Lactone.pdf

———. 2012. "Formal Recommendation: Glucono Delta Lactone." Washington DC: USDA-AMS-NOP. May 25, 2012. 3 pp.

OMRI. 2002. "Technical Advisory Panel Report for Glucono Delta Lactone." Washington DC: USDA-AMS-NOP. 16 pp.

———. 2015. "Technical Report on Citric Acid and its Salts." Washington DC: USDA-AMS-NOP. 31 pp. http://www.ams.usda.gov/sites/default/files/media/Citric%20Acid%20TR%202015.pdf

Pasternak, R. "Process of Crystallizing Pure D-Glucono-Delta-Lactone." U.S. Patent No. 2102380A, December 14, 1937, Charles Pfizer Company.

Pazur, J.H., and K. Kleppe. "The Oxidation of Glucose and Related Compounds by Glucose Oxidase from *Aspergillus niger*." *Biochem*. 3, no. 4 (1964): 578-583.

PMP (PMP Fermentation Products). 2004. "Glucono Delta Lactone." PMP Factsheet, PMP Fermentation Products, 900 NE Adams St., Peoria, IL 61603. 7 pp.

Purane, N.K., S.K. Sharma, P.D. Salunkhe et al. "Gluconic Acid Production from Golden Syrup by *Aspergillus niger* Strain Using Semiautomatic Stirred-Tank Reactor. *J. Microb. Biochem. Technol*. 4, no. 4 (2012): 92-95.

Rohr, M., C.P. Kubicek and J. Kominek. 1983. "Gluconic acid." In: Volume 3 of H.J. Rehm and G. Reed, eds. *Biotechnology: A Comprehensive Treatise in 8 Volumes*. (Basel: Verlag Chemie), pp. 456-465 of 642 pp.

Ramachandran, S., P. Fontanille, A. Pandey et al. "Gluconic Acid: Properties, Applications, and Microbial Production." *Food Technol. Biotechnol*. 44, no. 2 (2006): 185-195.

Rankin, S.A., C.M. Chen, D. Sommer et al. 2006. "Mozzarella and Scamorza Cheese." In: Y.H. Hui ed. *Handbook of Food Science, Technology, and Engineering*, Volume 4 (Boca Raton: CRC/Taylor and Francis), pp. 150-1 to 150-11.

Rubico, S.M. and M.R. McDaniel. "Sensory Evaluation of Acids by Free-Choice Profiling." *Chem. Senses* 17, no. 3 (1992): 273-289.

Ruijter, G.J.G., C.P. Kubicek and J. Visser. 2002. "Production of Organic Acids by Fungi." In: H.D. Osiewacz, ed. *The Mycota X Industrial Applications*. (Berlin: Springer Verlag), pp. 213-230.

Santos, M.H.S. and J.T. Zarzo. "Glucono Delta Lactone and Citric Acid as Acidulants for Lowering the Heat Resistance of *Cloistridium sporogenes* PA 3679 in HTST Working Conditions." *Intl. J. Food Microbiol*. 25 (1995): 191-197.

Sanjay, K.R., R. Subramanian, A. Setnhil. "Use of Natural Coagulants of Plant Origin in Production of Soycurd (Tofu)." *Intl. J. Food Eng*. 4, no. 1 (2008): 1-13.

Schuster, E., N. D.-Coleman, J.C. Frisvad et al. "On the Safety of *Aspergillus niger*—a Review." *Appl. Microbiol. Biotechnol.* 59 (2002): 426-435.

Seiskari, P., Y.-Y. Linko and P. Linko. "Continuous Production of Gluconic Acid by Immobilized *Gluconobacter oxydans* Cell Bioreactor." *Appl. Microbiol. Biotechnol*. 21 (1985): 356-360.

Shen, C.F., L. de Man, R.I. Buzzell, and J.M. deMan. "Yield and Quality of Tofu as Affected by Soybean and Soymilk Characteristics: Glucono Delta Lactone Coagulant." *J. Food Sci.* 56, no. 1, (1991): 109-112.

Singh, O.V. and R. Kumar. "Biotechnological Production of Gluconic Acid: Future Implications." *Appl. Microbiol. Biotechnol*. 75 (2007): 713-715.

Soccol, C.R., L.P.S. Vandenberghe, C. Rodrigues et al. "New Perspectives for Citric Acid Production and Application." *Food Technol. Biotechnol*. 44, no. 2, (2006): 141-149.

Stetten, M.R. and D. Stetten, Jr. "The Metabolism of Gluconic Acid." *J. Biol. Chem*. 187 (1950):241-252.

Stetten, M.R. and Y.J. Topper. "Pathways from Gluconic Acid to Glucose in Vivo." *J. Biol. Chem*. 203, no. 2 (1953): 653-664.

Stubbs, J.J., L.B. Lockwood, E.T. Roe et al. "Ketogluconic Acids from Glucose." *Ind. Eng. Chem*. 32, no. 12 (1940): 1626-1731.

Sun, N. and W.M. Breene. "Calcium Sulfate Concentration Influence on the Yield and Quality of Tofu from Five Soybean Varieties." *J. Food Sci*. 56, no. 6 (1991): 1604-1607.

Toldra, F. 2006. "Meat Fermentation." In: Y.H. Hui ed. *Handbook of Food Science, Technology, and Engineering*, Volume 4 (Boca Raton: CRC/Taylor and Francis), pp. 181-1 to 181-9.

Tsai, S.J., C.Y. Lan, C.S. Kao et al. "Studies on the Yield and Quality Characteristics of Tofu." *J. Food Sci*. 46 (1981): 1734-1740.

Tseng, R.Y.L., E. Smity-Nury and Y.S. Chang. "Calcium and Phosphorous Contents and Ratios in Tofu as Affected by the Coagulants Used." *Home Economics Res. J.* 6, no. 2 (1977): 171-175.

US EPA (United States Environmental Protection Agency). 2005. "Inert Tolerance Reassessment—Gluconic Acid and its Sodium Salt." USEPA, Washington, DC. 24 pp. http://www.epa.gov/opprd001/inerts/gluconicacid.pdf

US NIEHS (National Institute of Environmental Health Services). 2015. "Glucono Delta Lactone M010024 NTP."

USDA Agricultural Marketing Service. 2000. "National Organic Program Final Rule." *Federal Register* 65(246): 80548-80684. Washington DC: USDA-AMS-NOP. December 21, 2000. http://www.gpo.gov/fdsys/pkg/FR-2000-12-21/pdf/00-32257.pdf

———. 2003. "National Organic Program; Amendments to the National List of Allowed and Prohibited Substances." *Federal Register* 68(212): 62215-62218. Washington DC: USDA-AMS-NOP. November 3. www.gpo.gov/fdsys/pkg/FR-2003-11-03/html/03-27416.htm

———. 2008. "National Organic Program; Sunset Review 2008." *Federal Register* 73(197): 59479-59482. Washington DC: USDA-AMS-NOP. October 9. http://www.gpo.gov/fdsys/pkg/FR-2008-10-09/pdf/E8-24114.pdf

———. 2013. "National Organic Program; Sunset Review 2013." *Federal Register* 78,(192): 61154-61161. Washington DC: USDA-AMS-NOP. October 3. http://www.gpo.gov/fdsys/pkg/FR-2008-10-09/pdf/E8-24114.pdf

USDA FSIS (United States Department of Agriculture, Food Safety and Inspection Service). 2008. "False or Misleading Labeling or Practices Generally." (9CFR 317.8) http://www.gpo.gov/fdsys/pkg/CFR-2008-title9-vol2/pdf/CFR-2008-title9-vol2-sec317-8.pdf

UNEP (United Nations Environment Program). 2006. "SIDS Initial Assessment Report, Gluconic Acid and its Derivatives." UNEP Publications, Nairobi, Kenya. 231 pp.

Van Dijck, P.W.M., G.C.M. Selten and R.A. Hempenius. "On the Safety of a New Generation of DSM *Aspergillus niger* production strains." *Reg. Toxicol. Pharmacol*. 38 (2003): 27-35.

Wong, C.M., K.H. Wong and X.D. Chen. "Glucose Oxidase: Natural Occurrence, Function, Properties, and Industrial Applications." *Appl. Microbiol. Biotechnol*. 78 (2008): 927-938.

Xavier, N.M., A.P. Rauter, Y. Queneau. "Carbohydrate-Based Lactones: Synthesis and Applications." *Top. Curr. Chem.* 295 (2010): 19-62.

Yang, A. and A.T. James. "Effects of Soybean Protein Composition and Processing Conditions on Silken Tofu Properties." *J. Sci. Food Agric*. 93 (2013): 3065-3071.

Yilmaz, M.T. and O. Zorba. "Response Surface Methodology Study on the Possibility of Nitrite Reduction by Glucono Delta Lactone and Ascorbic Acid in Turkish Type Fermented Sausage (Sucuk). *J. Muscle Foods* 21 (2010): 15-30.