Glucono Delta-Lactone

Processing

Executive Summary

Glucono delta-lactone (GDL) was petitioned to be added to the National List as a tofu coagulant. It is produced by the oxidation of gluconic acid by a number of various methods. In addition to coagulation, GDL is used as an acidulant, leavening agent, and sequestrant. The NOSB was petitioned for this substance in 1995, and declined to refer it to the Technical Advisory Panel. The reviewers all considered it possible to make GDL from non-synthetic sources, although one considered certain sources and processes synthetic. All considered it to be non-agricultural. Two recommended that it be added to the National List with an annotation; one recommended that it remain off the National List. All three recommended that it be allowed for use in a made with organic (specified ingredients) claim. Of these, two supported with annotations, and one recommended no annotations for this use. Further investigation may be required to determine if sources are produced by the use of genetic engineering.

Summary of Advised Recommendation

95% organic

Synthetic / Non-Synthetic:	Allowed or Prohibited:	Suggested Annotation:
Either synthetic or non-synthetic depending on the source and manufacturing process (1) Non-synthetic (2)	Allowed (1) Prohibited (1)	(Reviewer 1) None. (Reviewer 2) Produced by microbial fermentation of carbohydrate substances or by enzymatic oxidation of organic glucose (Reviewer 3).Must be produced by fermentation of glucose by naturally occurring microorganisms or enzymes

Made with organic (70% or more organic ingredients)

Agricultural / Non-Agricultural	Allowed or Prohibited:	Suggested Annotation:
Non-agricultural (3)	Allowed (3) No (list votes)	(Reviewer 1) For tofu production only. GDL from fermentation of a non-GMO source of glucose with non-GMO microorganism or enzyme, and fermentation from a non-pathogenic microorganism. Level of GDL should be no more than the minimum amount to produce coagulation, in any case not to exceed 0.4% (Reviewer 2) Produced by microbial fermentation of carbohydrate substances or by enzymatic oxidation of organic glucose. (Reviewer 3)None.

Identification

Chemical Name(s): glucono delta-lactone

CAS Number: 90-80-2

Other Names: D-gluconic acid delta-lactone, D-glucono-1,5-lactone, Gluconolactone; delta-gluconolactone; GDL

Other Codes: EU No. E575

Characterization

Composition:

$C_6H_{10}O_6$

Properties:
A fine, white, practically odorless, crystalline powder. It is freely soluble in water and is sparingly soluble in alcohol. It decomposes at about 153°C (FCC, 1996). It hydrolyzes to gluconic acid in water.

How Made:
Glucono delta-lactone is prepared by direct crystallization from the aqueous solution of gluconic acid. Gluconic acid for food use in the US may be produced in any of three different ways (FDA, 21CFR184.1318):

1. by the oxidation of D-glucose with bromine water;
2. by the oxidation of D-glucose by microorganisms that are nonpathogenic and nontoxicogenic to man or other animals; or
3. by the oxidation of D-glucose with enzymes derived from these microorganisms.

Gluconic acid is customarily produced by fermentation using *Aspergillus niger*. The genes for glucose oxidase and catalase from *A. niger* have been isolated. Gluconic acid production can be improved with the use of cloned genes, and alternative economical host systems can be developed. (Nagarajan, 1992). Glucose oxidase-catalyzed oxidation of D-glucose is a method used in the commercial production of glucono delta-lactone (BeMiller, 1992).

Specific Uses:
Glucono delta-lactone is allowed for use in human food as a curing and pickling agent, a leavening agent, a pH control agent, and a sequestrant [21CFR184.1318]. The Merck Index cites use as a coagulant for tofu (Budavari, 1996). The TAP review will focus on use as a tofu coagulant, as per the petition's example, but other uses will be discussed to illustrate some specific functions and other characteristics of the material relative to the OFPA and NOSB criteria. Other uses in food processing include: acid (acidulant), leavening agent; and sequestrant (Food Chemicals Codex, 1996). Glucono delta-lactone may be employed as a chemical leavening agent, and has been used for instant bread that needs no proofing. Glucono delta-lactone is an inner ester of gluconic acid. In a water solution, GDL slowly hydrates to become acidic and thus acts as a leavening acid. Hydrolysis is slow in the cold but is accelerated by heat, making GDL ideal for refrigerated or frozen dough products. GDL's cost makes its use prohibitive in most baked goods, except in some refrigerated or frozen dough formulations (Chung, 1992).

Glucono delta-lactone is one of the less commonly used food acidulants. In conjunction with reducing compounds, GDL accelerates the rate of development of cure color in smoked meats, which considerably reduces the smoking time. (Friedman and Greenwald, 1992). The main function of curing accelerators is to accelerate color fixing or to preserve color of cured meat products during storage. Curing accelerators must be used only in combination with curing agents (Schmidt and Raharjo, 1992). The petitioned use is as a tofu coagulant.

Action:
Compared to other food acids, glucono delta-lactone has two differentiating properties.

Due to its slow hydrolysis to gluconic acid (which takes place gradually over the forty to sixty minutes after dissolution), it provides a gradual, progressive and continuous decrease of pH to equilibrium. Accordingly, it is used as a slow release acidulant. During its hydrolysis, its initial sweet taste becomes only slightly acidic, making the final flavor of an aqueous solution much less tart than that of other acidulants (Jungbunzlauer, 2002).

Traditional tofu making involves several steps. Soybeans are soaked, crushed, and cooked into a soymilk or 'slurry.' The coagulant is then added.

Historically, tofu coagulents were either a combination of magnesium chloride and magnesium sulfate extracted from seawater (nigari) or mined calcium sulfate (gypsum) (Shurtleff and Aoyi, 1975). Calcium chloride and GDL are also used. The gradual acidification produced by the GDL initiates the curdling of the protein. When glucono delta-lactone is used along with calcium chloride, these salts act to coagulate the soymilk into a silken smooth tofu. The coagulated tofu is then submerged in water, cut into blocks, and packed in water-filled tubs (Morinaga, 2002). Calcium sulfate and glucono delta-lactone are the coagulants of choice for tofu (Abd Karim et al., 1999).

Combinations:
Food grade glucono delta-lactone is usually sold as the pure material. The assay requirement for food grade material is not less than 99.0% and not more than 100.5% of $C_6H_{10}O_6$. Calcium sulfate and glucono delta-lactone are the coagulants of choice for tofu (Abd Karim et al., 1999). The petitioner indicates that they employ glucono delta-lactone as part of a proprietary combination of GDL, magnesium chloride and calcium sulfate.

Status

Historic Use:
GDL has not been historically listed in US or international certification standards. As of July 2002, it has been observed by a reviewer in the market place as a labeled ingredient in shelf stable (aseptic tetra pack) tofu products claiming "Made with organic ingredients" but not labeled as certified organic (identified as "Grown and handled in accordance with California Foods Act of 1990").

OFPA
Glucono delta-lactone is not specifically mentioned in the Organic Foods Production Act, and does not currently appear on the National List of Allowed Non-organic ingredients of the National Organic Program Final Rule (7 CFR 205.605).

The NOSB received a petition for GDL for use in soy products in 1995, but the NOSB chose to not refer that substance to the TAP (NOSB, 1995).

Regulatory
A Select Committee reviewed Glucono Delta-Lactone as a food ingredient and concluded "[t]here is no evidence in the available information on glucono delta-lactone that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future." (Life Sciences Research Office, 1981).

FDA:	21 CFR 184.1318	Direct Food Substance affirmed as GRAS
	21 CFR 133.129	Dry curd cottage cheese – optional acid ingredient
	21 CFR 155.120	Canned green beans and canned wax beans – optional acid ingredient
FSIS:	9 CFR 424.21	Ingredients allowed in meat and poultry products

European Parliament and Council Directive No 95/2/EC: GDL is listed as a generally permitted food additive (E575). It may be added to all foodstuffs, following the "quantum satis" principle, as long as no special regulation restricts the use.

EPA/NIEHS/Other Appropriate Sources
EPA - No information on the Envirofacts Master Chemical Integrator (EMCI) or Toxics Release Inventory (TRI) as of June 22, 2002.

NIEHS - No information in the National Toxicology Program (NTP) database for glucono delta-lactone as of June 22, 2002.

Other sources -
New Jersey Right-to-Know Hazardous Substance List – glucono delta-lactone is not listed.

Status Among U.S. Certifiers:
No certifiers list or refer to glucono delta lactone currently. *California Certified Organic Farmers (CCOF)* – CCOF Manual Two: USDA Requirements For Organic Producers (Dec. 2001) Not listed.

Oregon Tilth Certified Organic (OTCO) – Oregon Tilth Certified Organic Standards, Oct. 8, 2001. Not listed in Section 8, National List that accompanies the standards.

Organic Crop Improvement Association International (OCIA) OCIA Standards Manual NOP Standards plus OCIA International Requirements 2002: Not listed

Quality Assurance International (QAI) – Program Policies (web site July 2002) 5.2 Acceptable and Prohibited Materials - Until full implementation of the NOP, the general criteria used by QAI for determining the acceptability of materials is that specified by the Organic Materials Review Institute (OMRI). Clients will be notified of which materials they currently use that will not comply, upon full implementation of the NOP. All approvable materials must be on the National List for any product to be certified after October 21, 2002. [No specific reference to GDL.]

Texas Department of Agriculture (TDA) Organic Certification Program – TDA Organic Certification Program Materials List (February 2000) Not listed

Washington State Department of Agriculture (WSDA) Organic Food Program – Chapter 16-158-060 WAC (rev. January 18, 2001), not listed.

International
CODEX – Not listed.
EU 2092/91 – Not listed.
Japanese Agricultural Standard – Not listed.
IFOAM – Not listed.
Canada – Not listed.
Other International Certifiers – Could not find any that allow glucono-delta lactone for any purpose.

OFPA 2119(m) Criteria

(1) *The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems.* This is being considered as a processing material.

(2) *The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.* See processor criteria 3, below. Glucono delta-lactone is completely biodegradable.

(3) *The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.* This is considered below under item 2.

(4) *The effect of the substance on human health.* This is considered in the context of the effect on nutrition in 3, below, as well as the consideration of GRAS and residues in 5, below.

(5) *The effects of the substance on biological and chemical interactions in the agro-ecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.* As this is not released into the agro-ecosystem, there is no direct effect.

(6) *The alternatives to using the substance in terms of practices or other available materials.* See discussion of alternatives in 1, below.

(7) *Its compatibility with a system of sustainable agriculture.* This is considered more specifically below in the context of organic handling in 6, below.

Criteria from the February 10, 1999 NOSB Meeting

A PROCESSING AID OR ADJUVANT may be used if;

1. *It cannot be produced from a natural source and has no organic ingredients as substitutes.*
   Three main types of tofu are available in American grocery stores (Indiana Soybean Board, 1998).

- Firm tofu is dense and solid and holds up well in stir fry dishes, soups, or on the grill, where you want the tofu to maintain its shape. Firm tofu also is higher in protein, fat and calcium than other forms of tofu.
- Soft tofu is a good choice for recipes that call for blended tofu, or in Oriental soups.
- Silken tofu is made by a slightly different process that results in a creamy, custard-like product. Silken tofu works well in pureed or blended dishes. In Japan, silken tofu is enjoyed "as is," with a touch of soy sauce and topped with chopped scallions.

Different coagulants are used to make the different tofu types: calcium chloride for soft tofu (kori tofu); calcium sulfate, magnesium sulfate or magnesium chloride (with phosphoric or citric acid) for hard tofu; and glucono delta-lactone and calcium sulfate (alone or with GDL) for silken tofu (Saio, 1986). Glucono delta-lactone is used to make silken tofu, but it is not suitable for making firm (Chinese style) tofu if used alone (Tsai et al., 1981). Silken tofu can be made with calcium sulfate alone (Beddows CG and Wong J. 1987a, 1987b). However, comparing various coagulants for tofu, glucono delta-lactone yielded the best texture on the basis of smoothness (DeMan et al., 1986).

Glucono delta-lactone is produced by fermentation of glucose, a natural carbohydrate source. Glucono delta-lactone could be produced from organic glucose. It is possible to coagulate tofu with the use of other acidulants, such as organic vinegar or organic lemon juice. However, this results in a tart or sour tofu that is generally used as an intermediate for other products and that is seldom marketed at retail. Vinegar and lemon juice are primarily used in home recipes (Soyfoods Association, 1986).

2. *Its manufacture, use, and disposal do not have adverse effects on the environment and are done in a manner compatible with organic handling as described in section 6513 of the OFPA*

As indicated above, glucono delta-lactone is prepared by direct crystallization from an aqueous solution of gluconic acid. Gluconic acid may be produced by the oxidation of D-glucose with bromine water; by the oxidation of D-glucose (fermentation) by microorganisms that are nonpathogenic and nontoxicogenic to man or other animals; or by the oxidation of D-glucose with enzymes derived from these microorganisms.

Historically, gluconic acid was produced from glucose by direct oxidation with bromine water, a chemical process. Currently, glucono delta-lactone is customarily produced by fermentation of glucose, a natural carbohydrate source, by a naturally occurring microorganism, *A. niger*. Fermentation by natural microorganisms is a processing activity permitted in the OFPA. Glucose oxidase-catalyzed oxidation of D-glucose is a method used in the commercial production of glucono delta-lactone (BeMiller, 1992). The genes for glucose oxidase and catalase from *A. niger* have been isolated. Gluconic acid production can be improved with the use of cloned genes, and alternative economical host systems can be developed. (Nagarajan, 1992).

Glucono delta-lactone is available as a powder, which means that dust is an environmental hazard that must be managed in the normal course of use and disposal.

<u>Exposure Limits</u>
Expressed as Time Weighted Averages (TWAs) over 8 hour working shifts.

NIOSH Recommended Exposure Limit (REL) (guideline)
Total dust: 10 mg/m^3
Respirable fraction: 5 mg/m^3
Source: NIOSH, 1992

OSHA Permissible Exposure Levels (PEL) (regulation)
Total Dust: 15 ppm
Respirable fraction: 5 mg/m^3
No limits on skin exposure
Source: 29 CFR 1910.1000 (2000)

3. *If the nutritional quality of the food is maintained and the material itself or its breakdown products do not have adverse effects on human health as defined by applicable Federal regulations.*

The nutritional profiles of tofu made from various coagulation agents compares favorably for some nutrients and with lower nutritional value for others. Tofu made with glucono delta-lactone and calcium sulfate as a coagulant will have a higher calcium content and a lower magnesium content than tofu made with nigari (Soyfoods Association,

1986). Tofu made with glucono delta-lactone alone (no calcium salt) has a lower calcium-to-phosphorus ratio and a lower calcium content than a tofu made with a calcium salt (Tseng et al., 1977).
Glucono delta lactone, in an aqueous solution, forms an equilibrium mixture with its hydrolysis product, gluconic acid. These are intermediates in the oxidation of glucose through the pentose phosphate cycle, which, while not the main pathway of glucose metabolism, is well recognized.

4. *Its primary purpose is not as a preservative or used only to recreate/improve flavors, colors, textures, or nutritive value lost during processing except in the latter case as required by law.*
GDL's primary purpose is as a tofu coagulant. Coagulation of the soy proteins is an essential step that transforms soymilk into tofu. About half the protein of soy is soluble at neutral pH but insoluble in acid conditions or in the presence of alkaline elements such as calcium and magnesium. The gradual acidification produced by the GDL initiates the curdling of the protein. When glucono delta-lactone is used along with calcium chloride, these salts act to coagulate the soymilk into a silken smooth tofu.

While texture is one of the most striking characteristics that distinguishes GDL coagulated tofu from other coagulants, it is not solely used for texture. The coagulated tofu is then submerged in water, cut into blocks, and packed in water-filled tubs (Morinaga, 2002). Calcium sulfate and glucono delta-lactone are the coagulants of choice for tofu according to Abd Karim et al. (1999). Glucono delta-lactone is not used in tofu as a preservative. GDL is not used to recreate / improve flavors, colors, or nutritive value.

5. *Is Generally Recognized as Safe (GRAS) by FDA when used in accordance with Good Manufacturing Practices (GMP), and contains no residues of heavy metals or other contaminants in excess of FDA tolerances.*
Glucono delta-lactone has been affirmed by the FDA as Generally Recognized As Safe [21 CFR 184.1318] following a comprehensive review of the health aspects of glucono delta-lactone as a food ingredient in 1981 (Life Sciences Research Office, 1981)..

The FDA permits the uses described in Table 1.

Table 1
FDA Approved Uses of Glucono delta-lactone

Use	**21 CFR**
curing and pickling agent	170.3(o)(5)
leavening agent	170.3(o)(17)
pH control agent	170.3(o)(23)
sequestrant	170.3(o)(26)
Source: 21 CFR 184.1318(c)(1)	

The Food Chemicals Codex specifications for food grade glucono delta-lactone are:
Assay: Not less than 99% and not more than 100.5% of $C_6H_{10}O_6$.
Heavy metals: not more than 0.002% (20 mg/kg) expressed as lead.
Lead: not more than 10 mg/kg.
Reducing substances (as D-glucose): Not more than 0.5%.

6. *Its use is compatible with the principles of organic handling.*
Glucono delta-lactone has long been used by "silken tofu" makers.

Glucono delta-lactone is crystallized from an aqueous solution of gluconic acid. Currently, gluconic acid is customarily produced by fermentation of glucose, a natural carbohydrate source, by a naturally occurring microorganism, *A. niger.* Fermentation by natural microorganisms is a processing activity permitted in the OFPA. Glucono delta-lactone produced by microbial fermentation of glucose is analogous to citric acid produced by microbial fermentation of carbohydrate substrate (glucose), which is allowed in organic handling as an allowed nonagricultural non-synthetic substance [§ 205.605(a)(ii)].

Gluconic acid also is produced commercially by glucose oxidase-catalyzed oxidation of D-glucose (BeMiller, 1992). Gluconic acid production can be improved with the use of cloned genes, and alternative economical host systems can be developed. (Nagarajan, 1992). Thus, enzymes derived from GMO organisms may be involved in this process.

Glucose, the substrate for gluconic acid production, is allowed in organic livestock production as an ingredient of an applicable medical treatment [§ 205.603(6)].

7. *There is no other way to produce a similar product without its use and it is used in the minimum quantity required to achieve the process.* Other coagulants (calcium sulfate alone, calcium chloride, nigari) produce tofu with different physical and sensory characteristics (deMan et al., 1986). GDL is not considered appropriate by itself to make Chinese-style tofu (Tsai et al., 1981). It is possible to obtain a tofu with somewhat similar characteristics by the addition of carrageenan along with calcium sulfate or calcium acetate (Abd Karim et al., 1999), although the higher yield of tofu with glucono delta-lactone was not improved by adding carrageenan. Note that Abd Karim et al. (1999) state that calcium sulfate and glucono delta-lactone are the coagulants of choice for tofu. Industry standards for grades and quality of tofu are described in a publication from the Soyfoods Association (1986).

   A soft tofu can be produced using calcium sulfate or magnesium sulfate / magnesium chloride by the adjustment of stirring times and speeds (Hou, Chang, and Shih, 1997). GDL is used in silken tofu at a level of about 0.4%. Abd Karim et al. used 0.02 moles per liter, equivalent to 0.36%. DeMan et al. cite a range of 0.3% to 0.4%. Tsai et al. studied levels up to 0.08 N, equivalent to 1.4%, and found that a sour taste developed at concentrations greater than 0.025N, equivalent to 0.44%. Thus, the use of GDL is limited by its taste as well as its cost.

   Other acidulants possibly useful in organic tofu manufacture would be vinegar and lemon juice. These sources lack some of the properties (gradual development of acidity, milder taste) of glucono delta-lactone and are cited as being too sour. The sour taste of food acids restricts use of acidulants to the minimum quantity required to make the product.

TAP Reviewer Discussion[1]

Reviewer 1

[Ph.D food chemist with experience in organic certification]

. . . From the literature provided in the TAP review document, quantities/concentrations of GDL in tofu production are 0.01 to 0.08 **N** (Tsai et al., 1981), 0.3 to 0.4**%** (deMan et al., 1986), or 0.02 mol/L (Karim et al., 1999). These figures suggest that in practice there may be considerable latitude in the actual use of GDL in production. Since "The dose makes the poison", I would further recommend that an upper limit of GDL concentration in tofu be specified in the criterion, perhaps based on some fraction (e.g. the 5% EPA tolerance rule?) of the maximum GDL levels seen in representative tofu manufacture, or that specified in applicable regulatory Codices. . .

Sources and Substitutes

There are numerous papers and patents on the manufacture of GDL, obtained from oxidation of glucose by various potent oxidants. Such oxidants include sodium hypochlorite (Xie et al., 2000) and bromine (cited in the TAP review document, but references not found). Further, metallic catalysts such as platinum (Popvic and Tripkovic, 1996; Ashasi Chemical Industry Co. Ltd, Japan. 1980) and palladium (Chen et al., 1990; Ashasi Chemical Industry Co. Ltd, Japan, 1980) have been studied and patented specifically for production of GDL. All such non-biological oxidation reactions are purely synthetic and hence result in a synthetic product. Additionally, a patent was found describing the pure chemical synthesis of GDL from calcium gluconate (Kiersznicki et al., 1976).

Oxidation by fermentation of glucose by a naturally occurring fungus, Aspergillus niger to GDL qualifies as organic under NOSB criteria, provided that A. niger has not been genetically engineered and that it is non-pathogenic. [The reviewer found English abstracts for Japanese and Chinese patents to produce] . . . GDL by fermentation with A. niger (Gan et al., 1997) or with glucose-oxidase enzyme (Ube Industries, Ltd. Japan, 1982; Daicel Chemical Industries Ltd. Japan. 1985). . . [These patents did not indicate whether or not the fermentation organism or the enzyme were genetically engineered].

. . . Aspergillus niger is able to produce a mycotoxin—ochratoxin A—that poses health concerns to human and animals (Abarca et al., 2001). Therefore, if added to the NOP list, the source of GDL by fermentation should be very carefully monitored and scrutinized to be 1) from a non-GMO source, and 2) from an A. niger not producing any toxin or having any other pathogenic potential.

[1] *Any editorial additions are enclosed in square brackets in italics. Where a reviewer corrected a technical point these corrections were made in this document and are not listed here in the Reviewer Comments.*

The crystallization process of GDL from gluconic acid should be investigated. Crystallization processes may involve prohibited solvents. This reviewer found one patent that seems to be from an aqueous liquor (Leleu and Lemay, 1986) and thus unlikely to be of concern. . .

Manufacture, Use, and Disposal

Oxidation of glucose by bromine could produce side reactions of various brominated compounds. Such compounds may be potential toxic contaminants in GDL, or if removed from GDL, would be potential hazardous wastes.

The use of genetically modified organisms such as a modified *A. niger* for fermentation, or enzymes derived from genetically modified organism is not compatible with organic handling. . .

Human Health

The first sentence (The nutritional profiles of tofu…) suggests that in addition to increasing some nutritional qualities, tofu coagulants may decrease some others. Before approval, sufficient research should be available to more broadly examine these issues. For example, the addition of lime to milled corn (such as corn tortillas) is clearly beneficial from a calcium standpoint, but has been reported to increase the formation of toxic amino acid derivatives (lysinoalanine) on heating (Friedman, 1999a; 1999b). By analogy, it would be premature to assume that processing of tofu that enhances one nutrient may be without any adverse effects on other nutrient qualities. I would recommend that references to calcium are only tangential to the nutritional quality of tofu produced, and thus are irrelevant to the issue of approving GDL.

GDL has been shown to inhibit several enzymes of carbohydrate metabolism (Levvy et al., 1964; Palmer, 1971). While potentially inhibiting enzymes of carbohydrate metabolism, it appears that another study shows that at least GDL is sufficient to replace glucose for weight gain in rodents (Eyles and Lewis, 1943). However, such facile toxicology, while adequate in the 1943, may not be consistent with the intention of present-day organic standards.

The genotoxicity of GDL is apparently quite low, for example Ishidate et al. (1984) show that it caused no chromosomal structural aberrations. They did report a low level (1%) of polyploidy for gluconic acid exposure. Fukushima et al (1984) using the paramecium macronuclear assay concluded that GDL had some effect in the assay and needed further study. These studies at least suggest that further investigation might be warranted before approval for organic use.

One problem with using published toxicology for evaluating suitability for organic applications is that nearly all such research focuses on acute and high dose exposures. Example is this WHO Food Additive Series 21 report (attached). Chronic low dose toxicological data is very difficult to generate, especially for human subjects. Organic consumers rely on certified organic designations precisely to avoid being unwitting participants in unplanned, unintended and unmonitored chronic toxicology "experiments."

The fact that GDL rapidly equilibrates with gluconic acid in aqueous solution also makes it imperative that an evaluation of the potential toxicity of gluconic acid and its salts (e.g. calcium gluconate, magnesium gluconate and sodium gluconate) be conducted prior to any approval.

Primary Purpose

I do not understand the sentence "While texture is one of the most striking characteristics that distinguishes GDL coagulated tofu from other coagulants, it is not solely used for texture." Then what is the other purpose of GDL? All the documents provided with TAP review indicate that while tofu can be made with or without GDL, silken tofu requires GDL for its specific texture, and that hard tofu is better made without GDL. Therefore, GDL has a cosmetic effect under this use.

FDA status

It is clear in 7 CFR §205.671 that a "5% EPA tolerance" rule is recognized . . . that is that the normal regulatory tolerances are to be reduced by a factor of 20 from the levels acceptable for conventional agriculture *[65 Federal Register 80629]*. For organic to be meaningful this same standard must apply when setting tolerance levels with respect to other potentially toxic substances that may contaminate even GRAS food additives such as GDL. I recommend that in the event of approval of GDL that the FDA tolerances for lead or other contaminants be reduced in accordance with the above-mentioned "5% EPA tolerance" rule.

Compatibility

The last sentence "Glucose, the substrate…" should be amended to read "Synthetic glucose, the substrate…".

This argument, that synthetic glucose may be used in cattle production or in animal medical treatments, is not a basis for approving synthetic glucose for production of GDL for human consumption as an ingredient. This is too broad an interpretation of specific exceptions allowed in the CFR under certain narrow circumstances. In any case, there are available sources of non-synthetic glucose that can readily be used, from hydrolysis of starch for example (Budavari, 1996, 'Glucose' monograph). The issue of whether starch or cornstarch may be from GMO or GMO-contaminated crops is worthy of consideration here, since approval without reservation against such sources could further contribute to potential GMO damage to the consumer confidence in organic agriculture and/or organically labeled products.

Alternatives

Nigari (a by-product of traditional Japanese production of sea salt from sea water) [is one possible alternative].

Tofu is a traditional nevertheless somewhat synthetic food. Tofu manufacture using nigari or other natural coagulants has been done in Asia for perhaps two millennia (Tsai et al, 1981). It qualifies along with cheesemaking, brewing and bread baking as a traditional manufacturing process. As a food with a long and apparently benign history, we have little doubt that in this traditional form it would be inherently compatible with organic certification. The process of making tofu with GDL does not yet inspire the same confidence. Perhaps after some centuries this will change.

The last sentence "The sour taste of food…" suggests that acidulants such as vinegar and lemon juice will produce too much sourness in the final product. This problem can be addressed by addition of calcium hydroxide and/or magnesium carbonate (CFR §205.605 'allowed synthetics') during or after the coagulation step, effectively neutralizing the acidity while adding nutrients.

Conclusion

While GDL does not seem to have any obvious toxic effects, there are many uncertainties on how GDL is manufactured. If accepted, it should be subjected to at minimum the following restrictions:

1) it should be only from fermentation of glucose from a non-GMO source (no glucose from GMO corn for instance),
2) it should be fermented with *Aspergillus niger* or other microorganism that has not been genetically engineered,
3) the microorganism used in fermentation must be demonstrated to be not pathogenic and not producing any mycotoxin,
4) alternatively, GDL could be the product of oxidation by a non-engineered glucose oxidase
5) the crystallization process should not involve any prohibited substances.

This reviewer was unable to obtain sufficient information to confirm that any commercial source of GDL consistently meets all five of these criteria. Because of the nature of the product and the questions that remain, particularly with respect to imported sources, the NOSB should use caution in making a recommendation about this substance to the National List. Additionally, since gluconic acid is in an equilibrium state with GDL in aqueous solution, the suitability of gluconic acid and its salts must be considered by NOSB concomitant with GDL.

Since there are accepted alternatives to GDL for the production of tofu, this reviewer thinks it should <u>not</u> be added to the National list.

Recommendation Advised to the NOSB:

a. The substance is: Synthetic when produced by chemical catalytic oxidation of glucose, oxidation of glucose by genetically engineered enzyme, or fermentation of genetically engineered microorganism *[and]* Not Synthetic when produced by fermentation of non-GMO microorganism using a source of non-GMO glucose. *[It is]* Non-Agricultural.

b. in a product labeled 95% organic the substance should be Prohibited (do not add to National List).

c. in a product labeled "made with organic (specified ingredients)" The substance should be Allowed *only with the following additional restrictions suggested annotation*: For tofu production only. GDL from fermentation of a non-GMO source of glucose with non-GMO microorganism or enzyme, and fermentation from a non-pathogenic microorganism. Level of GDL should be no more than the minimum amount to produce coagulation, in any case not to exceed 0.4% (deMan et al., 1986).

<u>Reviewer 2</u>

[M.S. biochemistry. Research in food science with inspection, consulting, and certification experience. Western U.S.]

The packet of review materials gave a thorough survey of the literature and state of the science about GDL usage and tofu production. A minor point that was missing, but about which I could not find any good information, was what the estimated dietary intake of GDL might be if used in tofu production. There was information about consumer exposure in other products including "dairy product analogs" (which might be soymilk but I have no confirmation as to what that category really referred to) in the Life Sciences Research Office 1981 reference. The exposure levels listed were well below the levels used in feeding studies that showed no adverse effects. It was also noted that even this dietary exposure was likely high, because of the conversion of GDL to gluconate during processing.

Source and Substitutes

Glucono delta-lactone is produced by fermentation of glucose, a natural carbohydrate source. Glucono delta-lactone could be produced from organic glucose. It is possible to coagulate tofu with the use of other acidulants, such as organic vinegar or organic lemon juice. However, this results in a tart or sour tofu that is generally used as an intermediate for other products and that is seldom marketed at retail. Vinegar and lemon juice are primarily used in home recipes (Soyfoods Association, 1986).

The criteria listed discuss the production of tofu. The material being reviewed is GDL. As noted above, the sections discussing tofu should be deleted. The last paragraph (discussing GDL) should be the only one in this section. If this was the case, I would state that it can be produced from a natural source (fermentation is a natural and allowed process), but that it has no organic ingredients as substitutes (as noted in the last three sentences of the above paragraph).

It can be produced from a natural source. Fermentation is an allowable process as would be enzymatic oxidation of organic glucose. The potential organic acidulants (lemon juice or vinegar) aren't direct or suitable substitutes. The addition of calcium or magnesium salts that are currently on the NL can assist in the coagulation of proteins, but only in conjunction with a suitable acidulant.

Manufacture, Use, and Disposal

Some of the production methods would not be compatible with OFPA. An annotation regarding the production methods, similar to that specified for citric acid should be included. It would not appear to have any adverse impacts and to be compatible with organic handling practices.

Human Health

It would not appear to have any adverse effects on human health.

Primary Purpose

It is not a preservative and is not used to recreate or improve flavor, color, texture or nutritive value lost during processing of the food. In this case it does create (not recreate) the texture and some of the nutritive value of the food.

FDA Status

GDL is GRAS.

Compatibility

Its use is compatible with organic handling.

Alternatives

While there are other ways cited that a similar product could possibly be produced, use of GDL would appear to be necessary to produce the silky form of tofu.

Conclusion

This material has been used for a long time in the production of silky tofu. There is no evidence that there are any harmful effects of its use. The fact that it was not listed on any of the certifier lists is likely an oversight that was never caught before. Had this substance come up for review a long ago, I suspect that it would have been approved for the uses currently being sought. It can be produced using acceptable methodology (fermentation) and has unique properties that make it the material of choice for the production of silky tofu.

Recommendation Advised to the NOSB:

a. The substance is: Not Synthetic *and* Non-Agricultural

b. in a product labeled 95% organic the substance should be Allowed *only with the following restrictions (annotation):* Produced by microbial fermentation of carbohydrate substances or by enzymatic oxidation of organic glucose.

c. in a product labeled "made with organic (specified ingredients)" The substances should be Allowed *only with the following additional restrictions (annotation)* same as above.

Reviewer 3

[PhD. Food science University Professor, Western U.S.]

Sources and Substitutes
According to the 1986 paper by J.M. deMan et. al., a comparative study of assessment of five coagulants on the texture of tofu showed that minimal textural difference was obtained in producing a tofu product of high bulk weight and smooth (silky) texture for a 0.75% $CaSO_4$ solution in comparison to a 0.4% solution of glucono delta lactone. The authors concluded that there were minimal texture differences in peak force, compression, and firmness in tofu made with 0.5 to 1.0% concentrations of calcium sulfate vs. 0.3-0.4% concentrations of glucono delta lactone. This evidence seems to suggest that there are minimal textural differences between tofu coagulated with $CaSO_4$ or glucono delta lactone.

Manufacture, Use, and Disposal
It appears to be technically possible to produce glucono delta lactone by naturally occurring microorganisms. If the source of glucose-oxidase used to oxidize glucose to glucono delta lactone can be obtained from a non-GMO source, then commercial manufacture may be compatible and consistent with organic handling requirements.

Nutritional Quality
Review of the paper by R. Y. L. Tseng et al indicated that calcium content of tofu coagulated by calcium sulfate contained 170-208 mg calcium per 100 grams of tofu, while the calcium content of tofu coagulated with glucono delta lactone ranged from 39-41 mg./100 grams of tofu. This data suggests that tofu produced from glucono delta lactone was approximately four to five times lower in dietary calcium. An additional higher 2.0 – 2.5 to 1 Ca/P ratio was achieved with calcium sulfate coagulant in comparison to the 1.0 - 1.1 to 1 ratio for glucono delta lactone. It seems that use of calcium sulfate may provide significant nutritional advantages.

Compatibility with Organic Handling
Glucono delta lactone can be produced in a manner consistent with previous NOP approval of citric acid. If glucono delta lactone can be produced by microbial fermentation of glucose or alternatively by non-GMO glucose oxidase catalyzed oxidation of glucose, then its usage would be compatible with the principles of organic handling.

Alternatives
The primary advantage of using glucono delta lactone as a coagulant in tofu production is primarily its ability to produce a silky smooth texture form of tofu which has sensory advantages. However, the literature (i.e. J. M. deMan, et. al.) suggest that a 0.75% solution of calcium sulfate also produces a tofu product that provides a silken texture. Therefore use of calcium sulfate may provide an alternative unless there are textural changes that occur in the tofu as a function of scale up manufacturing conditions. The petitioner may wish to provide additional information relative to this issue.

Conclusion
Glucono delta lactone if manufactured by methods employing synthetic substrates or use of genetically altered cultures of microorganisms or enzymes or chemical modification of glucose should be prohibited. However, if glucono delta lactone can be manufactured as a commercial product utilizing by fermentation of glucose by naturally occurring microorganisms and/or enzymes such as glucose oxidase then a strong argument can be made to approve GDL as an allowed non-agricultural non-synthetic ingredient for "silken tofu" production.

Recommendation Advised to the NOSB:
a. The substance is: Not synthetic, Non-agricultural

b. in a product labeled 95% organic the substance should be Allowed only with *[the following]* suggested annotation: <u>Must be produced by fermentation of glucose by naturally occurring microorganisms or enzymes.</u>

c. in a product labeled "made with organic (specified ingredients)" the substances should be Allowed without further restriction

**** Additional Questions for Reviewers:

1) What are the qualitative differences between tofu made with glucono delta-lactone, (organic) vinegar, (organic) lemon juice, and other NOP-allowed food acids (citric acid or lactic acid)?
Reviewer 1
The reviewer is not a processor of tofu; please refer to the published literature to answer this question.

Reviewer 2
Tofu made with GDL has a different and unique texture. The other acidulants can coagulate proteins and form tofu, but the texture is not the same. From the materials available for review it is unclear whether a suitable combination of (organic) acidulants and (acceptable) calcium or magnesium salts might be capable of reproducing the silky tofu that is produced by GDL. I suspect not, or it would have been noted in some of the review articles, but there didn't appear to be any concerted effort to find conditions that duplicated the product produced using GDL.

Reviewer 3
Review of the papers of J.M. de Man *et al.* and H. J. Hou *et al.* provided in the review materials indicated significant differences in texture among coagulants used to produce commercial tofu products. Tofu produced from calcium chloride and magnesium chloride coagulated the soymilk instantly and gave a tofu that was coarse, granular and hard. Glucono delta lactone and calcium sulfate coagulated soymilk slowly but provided soft, smooth and uniform tofu with high bulk weight. Use of a weak acid such as vinegar (acetic acid), lemon juice (citric acid) and lactic acid would also coagulate soy protein from soymilk as a function of pH and provide more of a coarse coagulum. Additionally, use of these organic acids (i.e., acetic, citric and lactic) may contribute volatile flavor characteristics which may be undesirable in the product.

Manufacturing conditions can also effect quality characteristics of tofu. Therefore optimization of any commercial manufacturing operation must include not only the type of coagulant but also process operations optimization.

2) Is GDL produced from gluconic acid produced by glucose fermentation with non-genetically engineered organisms a "non-synthetic" material?
Reviewer 1
YES, provided glucose is from a non-GMO source.

Reviewer 2
It should be considered a non-synthetic material. I would argue for a GDL status similar to that of citric acid. Allowed as a non-synthetic with annotations that it must be produced by fermentation.

Reviewer 3
Production of GDL by microbial fermentation of non-GMO sources of glucose using non-GMO or natural microorganisms or extra-cellular enzymes would be considered non-synthetic and non-agricultural.

3) Is GDL produced from gluconic acid produced by glucose oxidation by bromine water, by fermentation with genetically engineered organisms, or by use of glucose oxidase enzyme expressed by a genetically engineered organism a synthetic material?
Reviewer 1
YES, definitely.

Reviewer 2
While it hasn't changed the nature of the material, these should be considered as prohibited practices and the use of GDL produced by these means should not be allowed for organic processing.

Reviewer 3
All of these conditions would produce a GDL product that would be synthetic and would be inconsistent with the basic principles of organic integrity.

4) Is use of GMO-origin enzymes common for GDL production?
Reviewer 1
The reviewer does not know. This information is likely to be proprietary to the manufacturers and should be subject to specific restrictions for any GDL approval.

Reviewer 2

It would appear from the review materials available for review that GMO enzymes are not currently in common use for the production of GDL. It would also appear that the possibility for this to change is quite high as the enzymes critical for GDL production have been identified.

Reviewer 3
I am not sure. I would highly recommend that the petitioner provide documentation from their supplier and/or manufacturer of the GDL as to the GMO status of the microorganisms and/or enzymes, which may be used in the process. I have not been able to locate any scientific papers that focus on commercial GDL manufacture.

5) Can certifiers and processors distinguish the naturally fermented glucono delta-lactone from the other sources?
Reviewer 1
NO, not without some sort of traceable certification. It may be possible to uncover GDL produced by bromination if a brominated compound is found as a residual contaminant. However, it is unlikely that a metal catalyzed production would be easily detected. It is not possible to distinguish a GDL that was fermented with a GMO organism, or produced with a GMO enzyme. Since GDL is purified from the microorganism or the enzyme, there is no biochemical assay such as PCR-based methods to uncover the use of a GMO organism.

Reviewer 2
Not likely by any end product testing. Certifiers would have to rely on documentation from the suppliers that GDL had been produced in a manner compatible with organic regulations.

Reviewer 3
As both a processor inspector and reviewer for West Coast certification agencies, certifiers could definitely distinguish naturally fermented glucono delta lactone from other sources by requiring the following documentation.
1. A statement from the manufacturer that no GMO microorganisms are used in its manufacture.
2. Produced by microbial fermentation of carbohydrate substances.

6) Has any company commercialized a GMO source of gluconic acid?
Reviewer 1
Unknown to the reviewer.

Reviewer 2
Not that I am aware of.

Reviewer 3
I am not aware of any commercialized GMO source of gluconic acid.

7) Is there any correlation between the use of GDL in shelf-stable tofu and superior keeping (preservation) qualities? Is there a reason why GDL is needed in the shelf stable packages?
Reviewer 1
It was not mentioned in the TAP review document that GDL had an additional role of preserving tofu. However, the gluconic acid released by GDL would restrict microbial growth by lowering the pH. Example of such phenomenon was measured in cottage cheese made with gluconic acid (el-Shenawy and Marth, 1990).

Reviewer 2
GDL is not needed to improve the keeping qualities of tofu. GDL is not used as a preservative, but is necessary for the precipitation of proteins which turns liquid soymilk into solid tofu. While other acidulants can serve this function the unique properties of GDL make it the material of choice for the manufacturing of silky tofu.

Reviewer 3
Use of GDL has been shown to increase moisture retention in tofu products especially in comparison to use of calcium sulfate as a coagulating medium. This effect may reduce the rate of syneresis (moisture migration out of the product) and create textural changes as well as noticeable loss of product quality (i.e. loss of silken texture). Please see Table 1 page 162 of the A. A. Karim *et al* reference provided in the review materials for additional moisture retention data.

8) If you believe GDL should be permitted, do you recommend any of the below options?
a) GDL should be added to the list at 205.605(a) as a "non-agricultural (non-organic) substance"? This is comparable to citric acid which is listed there with annotation- "produced by microbial fermentation of carbohydrate substances."

b) GDL should be considered approved under the provisions of 205.606 as an agricultural substance and required to be produced from organic glucose when available. This would mean that GDL would not appear on the List at 205.605 or explicitly at 205.606.

Reviewer 1
If GDL was approved under 205.606, restrictions about GMO and pathogenicity of the microorganism should be very clearly stated.

Reviewer 2
I would favor option a). I believe that it is compatible with organic processing and that a classification similar to that of citric is appropriate. It can (and should) be produced by similar technology, but because of possible changes in production techniques, it should have an annotation similar (or identical) to that used for citric acid.

Reviewer 3
I would recommend option A as I indicated in Part 4 of the formal review. In many respects review of GDL is similar to the issues of citric acid.

c) Any other suggestions as to how and where it should be listed?

Reviewer 1
GDL should be considered, and if listed, should be together with gluconic acid and its salts, sodium, potassium, calcium and magnesium gluconate.

Conclusion
Glucono delta-lactone is a non-agricultural additive that can be produced in a non-synthetic way. It is commonly used and preferred to make silken soft tofu. Reviewers believed that it should be added to the National List with annotations or not added to the National List at all for use in an 'organic' processed food product. All reviewers recommended that it be allowed for use in a made with organic (specified ingredients) claim. Of these, two reviewers supported with annotations, and one recommended no annotations for this use.

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