United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/national-list/petitioned

Document Type:

☐ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☒ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

Ascorbic Acid

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
Ascorbic Acid
L-Ascorbic Acid
(2*R*)-2-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one
L-Threoascorbic Acid

Other Names:
Vitamin C
Cevitamic Acid
Xyloascorbic Acid, L
Vitacimin
Vitacin
Ascoltin
Antiscorbutic factor

Trade Names:
Magnorbin
Ascorbicap
Hybrin
Cescorbat

CAS Numbers:
50-81-7

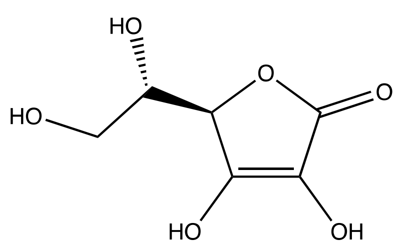
Other Codes:
EC No. 200-066-2
ICSC No. 0379
FEMA No. 2109
RTECS No. C17650000
UNII No. PQ6CK8PD0R

Summary of Petitioned Use

The United States Department of Agriculture (USDA) has approved synthetic sources of ascorbic acid as a "nonagricultural (nonorganic) substance allowed as an ingredient in or on processed products labeled as 'organic' or 'made with organic (specified ingredients or food group(s),'" at Title 7 of the Code of Federal Regulations (CFR) Section 205.605. Ascorbic acid is used in many handling and processing applications, including for nutritional fortification, and as an antioxidant, pro-oxidant, and preservative. This technical report was requested by the National Organic Standards Board (NOSB) Handling subcommittee in support of its sunset review of this listing.

Characterization of Petitioned Substance

Composition of the Substance:

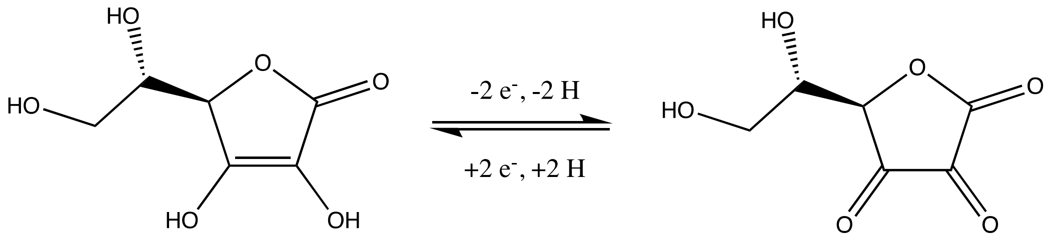


L-Ascorbic Acid

Figure 1

L-Ascorbic acid is a naturally occurring chiral vitamin, as shown in Figure 1. The opposite enantiomer of ascorbic acid (D-ascorbic acid), is not biologically active, and thus is not produced in the biosynthesis or in major industrial syntheses of ascorbic acid (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015). Because the D-form is missing in these relevant natural and industrial settings, the term "ascorbic acid" will refer exclusively to L-ascorbic acid throughout the remainder of this report.

Ascorbic acid is commercially available as a white to slightly yellow solid with ≥98% purity. Although ascorbic acid is the active species in vitamin C, the vitamin is formally composed of both ascorbic acid and its oxidized form, dehydroxyascorbic acid, as shown in the redox reaction in Equation 1 (Davey et al. 2000).



L-Ascorbic Acid | L-Dehydroxyascorbic Acid

Equation 1

Source or Origin of the Substance:
Ascorbic acid is the active substance in vitamin C and is naturally produced in nearly all plants and in many animal species (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, Gallie 2013). The biosynthesis of ascorbic acid was once thought to proceed through a carbon chain inversion of glucose; however, data compiled in recent studies are inconsistent with this mechanism (Isherwood et al. 1954, Braun et al. 1994, Banhegyi et al. 1997, Davey et al. 2000, Hancock and Viola 2005). Current work with *Arabidopsis* has shown support for several biosynthetic pathways for ascorbic acid production, which may be specific to the plant species and environmental conditions (Wolucka and Van Montagu 2003, Lorence et al. 2004, Hancock and Viola 2005). However, more research is required to establish the mechanisms of these syntheses (Davey et al. 2000, Wolucka and Van Montagu 2003, Lorence et al. 2004, Hancock and Viola 2005).

Industrially synthesized (synthetic) ascorbic acid follows a mechanism along the lines of the originally proposed biosynthetic pathway through the carbon chain inversion of glucose (Crawford 1982, USDA 1995a, EFSA 2013a, EFSA 2013b, EFSA 2015). The original synthesis of ascorbic acid was reported by Tadeus Reichstein in the 1930s, through what has become known as the Reichstein Process (Reichstein et al. 1934, Crawford 1982, Davey et al. 2000, EFSA 2013a). The Reichstein Process was the primary means of ascorbic acid production for more than 50 years before a commonly used, modern alternative that produces intermediates through fermentation processes replaced it (Davey et al. 2000, Oster and Fechtel 2012, EFSA 2013a, EFSA 2013b, EFSA 2015).

Properties of the Substance:
Ascorbic acid is a water-soluble vitamin with well-documented reducing power (Diplock et al. 1998, Davey et al. 2000, Timberlake 2015). This reducing power is the source of its biological importance as an enzymatic cofactor, pro-oxidant, and antioxidant (Jungeblut 1937, Uri 1961, Cort 1982, Frankel 1996, Diplock et al. 1998, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015).

Selected properties of ascorbic acid are listed in Table 1 on the following page.

Table 1: Properties of Ascorbic Acid

Property	Ascorbic Acid
Empirical Formula	$C_6H_8O_6$
Molecular Weight	176.12 g/mol
CAS No.	50-81-7
Appearance	White to slightly yellow crystals or powder
Melting Point	190–192^o C (decomposition)
Water Solubility	400 g/L (40^o C)
Density	1.65 g/cm^3
PKa	4.7 (10^o C)
Reactivity	Undergoes reactions in aqueous solution, upon exposure to a combination of air and light, bases, metals including copper and iron

Sources: PubChem 54670067, Uri 1961, O'Neil 2006, Lewis 2007, Lide 2007, Sigma-Aldrich 2014

Specific Uses of the Substance:

Ascorbic acid is the active compound in vitamin C and is produced in nearly all plants and most species of animals (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, Gallie 2013). Ascorbic acid is also an industrially produced commodity, with preparations from D-glucose via the Reichstein or adapted fermentation processes (Reichstein et al. 1934, Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015). Due to the availability of synthetic sources of ascorbic acid, it has become the primary source of the substance for food handling and processing applications.

Medicinal Applications

Ascorbic acid is the active compound in vitamin C, which provides many important biological functions. Ascorbic acid's ability to act as a reducing agent makes it an important cofactor, promoting enzymatic activity especially in the biosynthesis of the collagen protein (Levine and Hartzell 1987, Padh 1990, Ghosh et al. 1997, Jung and Wells 1997, Davey et al. 2000, Holst and Frolich 1907, Svirbely and Svent-Gyorgi 1932, Svirbely and Svent-Gyorgi 1933). While scurvy develops due to insufficient concentrations of ascorbic acid, the application of ascorbic acid may cure this disease (Holst and Frolich 1907, Svirbely and Svent-Gyorgi 1932, Svirbely and Svent-Gyorgi 1933, Davey et al. 2000).

The maintenance of enzymes with a low positive charge (Fe^{2+}, Cu^{+} vs. Fe^{3+}, Cu^{2+}) provides pro-oxidant characteristics, which have been correlated to improved immune responses (Duarte and Lunec 2005, Carocho and Ferreira 2013). In these circumstances, ascorbic acid donates an electron to Fe^{3+}, converting it to Fe^{2+}. The movement of electrons between the ascorbic acid and iron ion results in the formation of a reactive oxygen species (ROS), which can react with nearby biological systems (Davey et al. 2000, Duarte and Lunec 2005, Carocho and Ferreira 2013). The conditions that result in ROS formation are typically related to an immune response, where the ROS reacts with a pathogen such as a virus or bacteria (Davey et al. 2000, Duarte and Lunec 2005, Carocho and Ferreira 2013).

Preservative

Ascorbic acid is often included in food products as a preservative. Preservative applications of ascorbic acid make use of its antioxidant properties to prevent discoloration in a range of processed products and to protect from spoilage in lipid-based products (e.g., food oils) (Pizzocaro et al. 1993, Frankel 1996, Vermeiren et al. 1999, Choi et al. 2002, Tapia et al. 2008, Rojas-Grau et al. 2009). Ascorbic acid is most commonly used as a preservative to prevent enzymatic browning reactions that occur during processing and post-processing storage (Pizzocaro et al. 1993, Tapia et al. 2008, Rojas-Grau et al. 2009). Based on the relative instability of ascorbic acid, its function as a preservative is enhanced under acidic conditions (low pH) and at reduced temperatures (Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009).

Nutritional Fortification

There are several animals that have lost the ability to produce ascorbic acid (Chatterjee 1973, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015). These animals include humans, a variety of primates, and guinea pigs, all of whom must obtain ascorbic acid through their diets, making it an essential vitamin.

Since ascorbic acid is also water-soluble, it is unable to be stored in the body and, therefore, must be consumed daily (Timberlake 2015). Moreover, due to the relative instability of ascorbic acid (see reactivity in Table 1), its concentration is often reduced by the processing and storage of foodstuffs (Vermeiren et al. 1999, EFSA 2013b). Ascorbic acid is often added to processed foods because of daily dietary requirements and its relative instability (Frankel 1996, Vermeiren et al. 1999, Choi et al. 2002, Teucher et al. 2004, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015).

Approved Legal Uses of the Substance:
The USDA NOP has approved ascorbic acid as a synthetic "nonagricultural (nonorganic) substance allowed as an ingredient in or on processed products labeled as 'organic' or 'made with organic (specified ingredients or food group(s)'" at 7 CFR 205.605.

The United States Food and Drug Administration (FDA) has granted ascorbic acid GRAS (generally recognized as safe) status for use as a chemical preservative at 21 CFR 182.3013 and §582.3013 (for animal feeds), as well as for use in nutrients and dietary supplements at §182.8013 and §582.5013 (also for animal feeds). The FDA has approved ascorbic acid for use as a preservative in "frozen raw breaded shrimp," as a means "to retard development of dark spots on the shrimp," at §161.175. Ascorbic acid may be used as a dough conditioner in flour and whole wheat flour with the limitation that the "quantity not exceed 200 parts per million," at §137.105 and §137.200, respectively.

The FDA has also approved ascorbic acid as an ingredient in a range of canned food products. When used as an ingredient in canned peaches, apricots, and fruit cocktails, ascorbic acid must be added "in an amount no greater than necessary to preserve color," as stipulated at §145.170, §145.115, and §145.135, respectively. When used as an ingredient in canned mushrooms, the FDA limits the addition of "ascorbic acid (vitamin C) in a quantity not to exceed 132 milligrams for each 100 grams (37.5 milligrams for each ounce) of drained weight of mushrooms," at §155.201. When used in "canned artichokes packaged in glass containers," the substance "may be added in a quantity not to exceed 32 milligrams per 100 grams of finished food," as stipulated at §155.200. Section 155.200 also limits the amount of ascorbic acid added to canned asparagus to the "amount necessary to preserve color in the 'white' and 'green-tipped and white' color types." When added to canned applesauce, the FDA requires that the addition of "ascorbic acid as an antioxidant preservative in an amount not to exceed 150 parts per million; or ascorbic acid (vitamin C) in a quantity such that the total vitamin C in each 113 g (4 ounces) by weight of the finished food amounts to 60 mg," at §145.110. The USDA also allows the "addition of ascorbic acid or other preservatives to prevent oxidation of produce; butchering livestock and poultry; cleaning fish; and the pasteurization of milk," at 7 CFR 225.17.

The FDA has identified ascorbic acid as a required nutrient in infant formula, with a minimum level of 8 milligrams "for each 100 kilocalories of the infant formula in the form prepared for consumption," at 21 CFR 107.100. The FDA has approved ascorbic acid as an ingredient in "drug products containing active ingredients offered over-the-counter (OTC) for external use as hair growers or for hair loss prevention," at §310.527.

Ascorbic acid has been approved as a curing accelerator "to accelerate color fixing or preserve color during storage," in "cured pork and beef cuts, cured poultry, cured comminuted poultry and meat food products" at 9 CFR 424.21. Ascorbic acid has also been approved "to delay discoloration" in "fresh beef cuts, fresh lamb cuts, and fresh pork cuts" at §424.21.

Ascorbic acid has been approved for use in the formulation of wine and juice "to prevent oxidation of color and flavor components of juice and wine," and it "may be added to grapes, other fruit (including berries), and other primary wine making materials or to the juice of such materials, or to the wine, within the limitations which do not alter the class or type of the wine," at 27 CFR 24.246.

The United States Environmental Protection Agency (EPA) has identified ascorbic acid as "an inert or an active ingredient in a pesticide chemical formulation, including antimicrobial pesticide chemicals" that is "exempted from the requirement of a tolerance under FFDCA [Federal Food, Drug, and Cosmetic Act]

Section 408, if such use is in accordance with good agricultural or manufacturing processes" at 40 CFR 180.950. The EPA has also granted the substance an "exemption for pesticides of a character not requiring FIFRA [Federal Insecticide, Fungicide, and Rodenticide Act] regulation," at §152.25.

Action of the Substance:

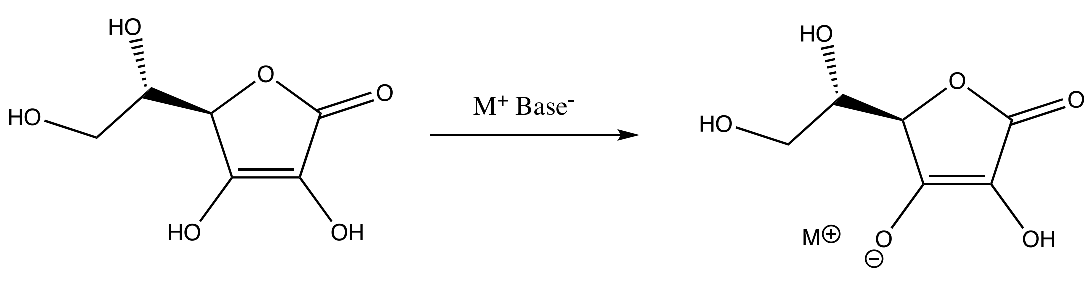
The activity of ascorbic acid is due to its ability to act as a reducing agent (that donates electrons) within biological settings (Timberlake 2015). This ability makes it an important enzymatic cofactor, which typically helps to promote enzymatic activity by maintaining the reduced form of iron and copper at the active site (Fe^{2+} and Cu^{+}, respectively) (Levine and Hartzell 1987, Padh 1990, Ghosh et al. 1997, Jung and Wells 1997, Davey et al. 2000). This cofactor activity has been reported for a range of enzymatic processes, although it is most widely known in the biosynthesis of the collagen protein (Holst and Frolich 1907, Svirbely and Svent-Gyorgi 1932, Svirbely and Svent-Gyorgi 1933, Davey et al. 2000). Ascorbic acid has been shown to promote the active Fe^{2+} state in iron dioxygenases that is essential for the crosslinking of the collagen helix (Padh 1990, Davey et al. 2000).

The reducing ability of ascorbic acid makes it an important biological antioxidant, providing protection from reactive oxygen species (ROS, molecules that remove electrons from nearby biological systems). (Simic and Karel 1980, Frankel 1996, Halliwell 1996, Davey et al. 2000, Carocho and Ferreira 2013). This protection is offered by the neutralization of the reactive species through electron transfer (redox reaction) (Timberlake 2015). In the absence of ascorbic acid or other antioxidants, ROS produce undesired modifications to biological structures such as DNA, RNA, proteins, and lipids, which may result in mutations (Lu et al. 2010, Craft et al. 2012, Carocho and Ferreira 2013, Timberlake 2015).

Ascorbic acid's antioxidant properties enable it to act as a scavenger of oxidized species, including radicals and dioxygen gas (O_2) (Vermeiren et al. 1999, Rojas-Grau et al. 2009). Oxidized species are responsible for the deterioration of a range of food products, including processed fruits, vegetables, meats, juice, wine, and beer, making their elimination by reaction with ascorbic acid (electron transfer) important for extending shelf-lives and protecting food products from undesirable tastes and odors (Pizzocaro et al. 1993, Vermeiren et al. 1999, Davey et al. 2000, Rojas-Grau et al. 2009).

Combinations of the Substance:

Ascorbic acid is commercially available in high purity (≥98%) and is often used as a preservative and/or antioxidant ingredient alone (Vermeiren et al. 1999, Rojas-Grau et al. 2009). However, due to its acidic nature (pKa = 4.7), it is physiologically present as its conjugate base (ascorbate salt), as shown in Equation 2 (Uri 1961, Davey et al. 2000, Timberlake 2015). Since ascorbic acid is biologically found as ascorbate anion, it may also be applied as a salt, rather than its neutral, acidic form (Davey et al. 2000, Teucher et al. 2004, EFSA 2013a, EFSA 2013b, EFSA 2015). These salts vary in the identity of the metal cation (M^{+} in Equation 2), although sodium (Na^{+}), calcium (Ca^{2+}), and iron ($Fe^{3+/2+}$) cations are common (Davey et al. 2000, Teucher et al. 2004, EFSA 2013a, EFSA 2013b, EFSA 2015).



L-Ascorbic Acid

L-Ascorbate Salt

Equation 2

Status

Historic Use:
Ascorbic acid has been historically used as a preservative, nutritional fortifier, and in medicine (USDA 1995a, Davey et al. 2000, EFSA 2015, Timberlake 2015). The earliest applications of ascorbic acid in modern medicine include treatments for scurvy, dietary supplements, and/or fortification to prevent the onset of the disease (Holst and Frolich 1907, Svirbely and Svent-Gyorgi 1932, Svirbely and Svent-Gyorgi 1933, Davey et al. 2000). Since these early findings, there has been a great deal of investigation on the effects of ascorbic acid on immune responses, from cold to cancer treatments (Jungeblut 1937, Davey et al. 2000, EFSA 2015, Timberlake 2015, Cho et al. 2018).

Ascorbic acid is often added to processed foods for nutritional purposes because humans need to consume it as a part of a daily diet and because of the water-soluble nature of ascorbic acid's metabolization (Chatterjee 1973, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015). However, its addition as a nutritional fortifier also provides preservative properties. The preservative nature of the compound is derived from its reducing nature, through which it reacts with oxidized species (including radicals and molecular oxygen) to prevent enzymatic browning and food spoilage (Gill and McGinnes 1995, Schozen et al. 1997, Berenzon and Saguy 1998, Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009, EFSA 2015).

Organic Foods Production Act, USDA Final Rule:
Ascorbic acid is not listed in the Organic Foods Production Act of 1990 (OFPA).

The USDA NOP has approved synthetic sources of ascorbic acid as a "nonagricultural (nonorganic) substance allowed as an ingredient in or on processed products labeled as 'organic' or 'made with organic (specified ingredients or food group(s)'" at 7 CFR 205.605(b). The USDA NOP has also approved synthetic ascorbic acid (Vitamin C) for use as "a plant or soil amendment for use in organic crop production," at §205.601(j).

International:

Canadian General Standards Board Permitted Substances List –
Ascorbic acid is listed in the Canadian General Standards Board Permitted Substances List (CAN/CGSB-32.311-2015) in Table 4.2 as allowed for "soil amendments and crop nutrition," and "synthetic and non-synthetic sources may be used as a pH regulator." Ascorbic acid is listed in Table 6.3 as an "ingredient classified as a food additive," and in Table 6.5 as a "processing aid for use as an anti-browning agent prior to the extraction or concentration of fruit or vegetable juice." Table 7.3 lists ascorbic acid as a "food-grade cleaner, disinfectant, and sanitizer permitted without a mandatory removal event."

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999) –
Ascorbic acid is listed in the CODEX (GL 32-1999) in Table 3.1 as a "food additive, including carriers."

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008 –
Ascorbic acid is not listed in EC No. 834-151 2007.

Ascorbic acid is listed in EC No. 889/2008 as a "food additive, including carriers," and is approved for "preparation of foodstuffs of plant origin and animal origin."

Japan Agricultural Standard (JAS) for Organic Production –
Ascorbic acid is listed in the JAS for Organic Production Notification No. 1606 as a "food additive, limited to be used for processed foods of plant origin."

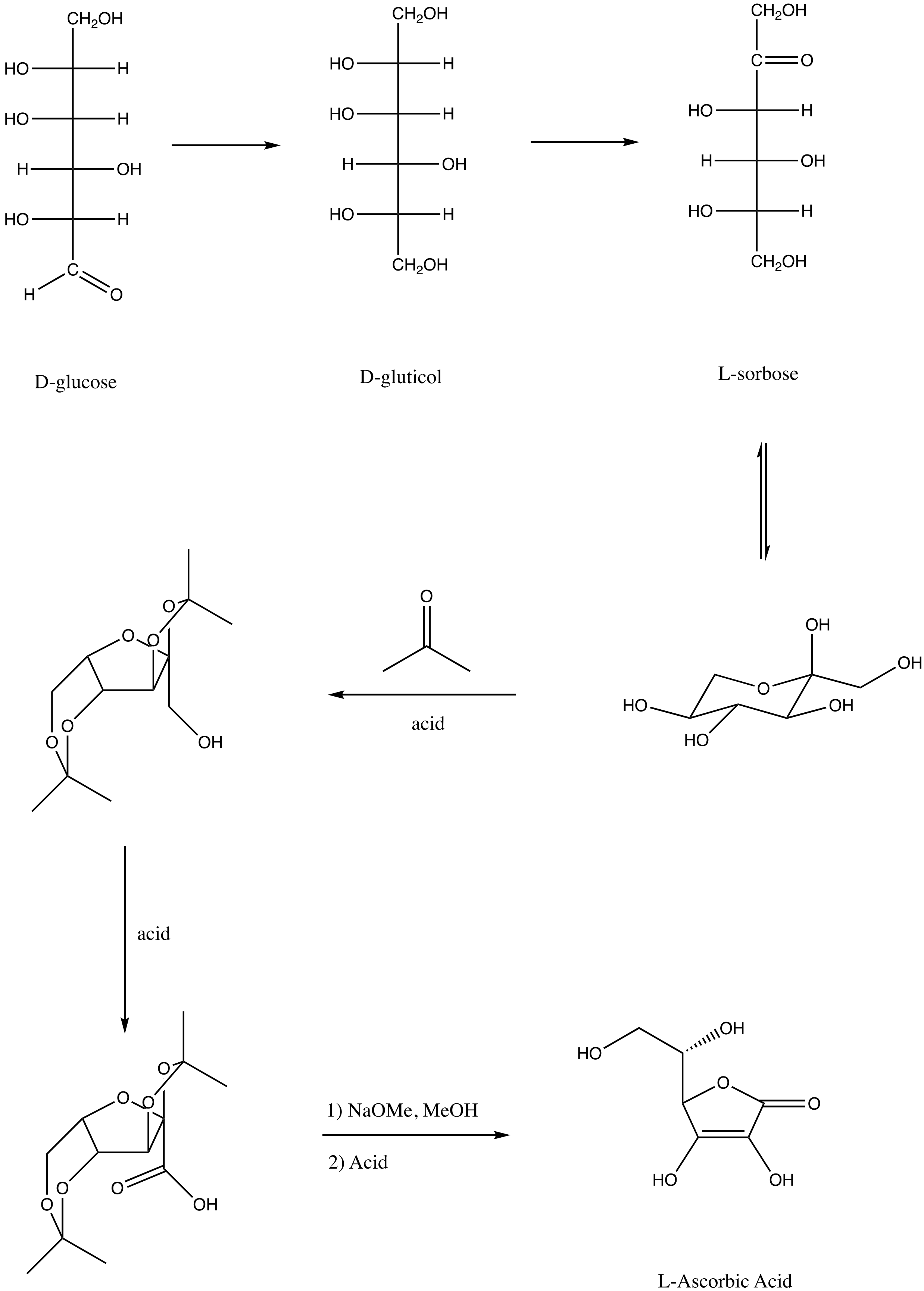
International Federation of Organic Agriculture Movements (IFOAM) –
Ascorbic acid is listed in IFOAM as an "approved additive and processing/post-harvest handling aid."

Evaluation Questions for Substances to be used in Organic Handling

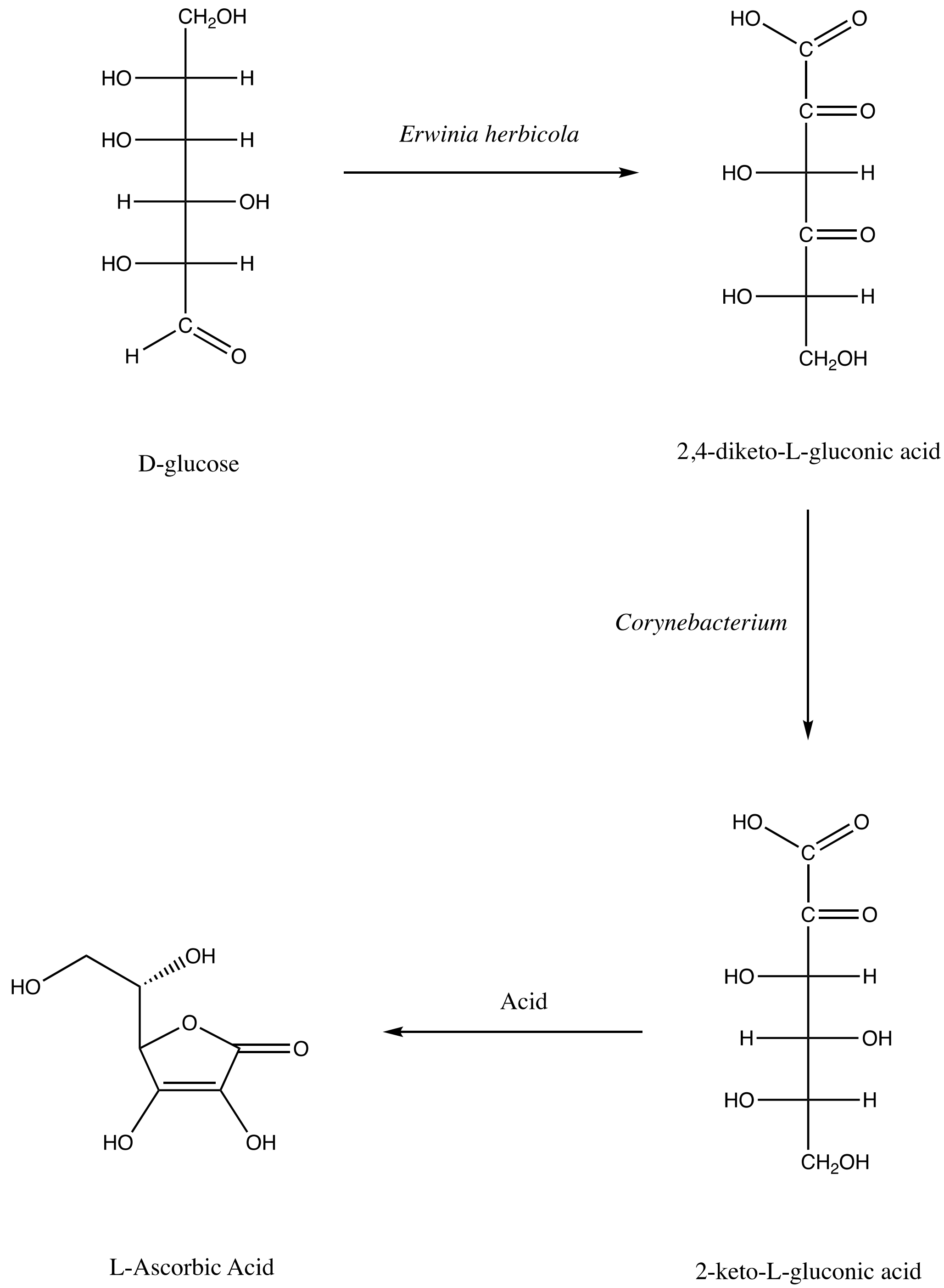
Evaluation Question #1: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant,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

Industrially synthesized (synthetic) ascorbic acid follows a mechanism along the lines of the originally proposed biosynthetic pathway through the carbon chain inversion of glucose (Crawford 1982, EFSA 2013a, EFSA 2013b, EFSA 2015). The original synthesis of ascorbic acid was reported by Tadeus Reichstein in the 1930s, through what has become known as the Reichstein Process (Reichstein et al. 1934, Crawford 1982, Davey et al. 2000, EFSA 2013a). This synthesis from D-glucose begins with a carbon chain inversion and reduction to D-gluticol, and subsequent oxidation to L-sorbose via fermentation, as shown with the Fischer projections (linear structures in Equation 3 on the following page) (Crawford et al. 1982). Like all sugar molecules, sorbose freely converts between linear and ring forms and, through this constant equilibrium, the ring hydroxyl groups are protected in a reaction with acetone in the presence of acid. This yields the multicyclic structure shown in Equation 3 (Crawford 1982, Timberlake 2015). The protected multicyclic structure is oxidized to a carboxylic acid at the only available alcohol position. The protected multicyclic carboxylic acid undergoes deprotection and conversion to ascorbic acid by stepwise treatment with sodium methoxide in methanol, followed by acidification (Crawford 1982). In the years following the initial report, reaction conditions for individual steps were optimized to improve yields and provide milder reaction conditions, although the steps and intermediates remained largely unchanged (Crawford 1982). The Reichstein Process was the primary means of ascorbic acid production for more than 50 years (Crawford 1982, Davey et al. 2000).

Despite the effectiveness of the purely synthetic production of ascorbic acid with the Reichstein Process, most modern industrial production of ascorbic acid employs fermentation processes (USDA 1995a, Davey et al. 2000, EFSA 2013a, Witcoff et al. 2013, EFSA 2015). The fermentative production of ascorbic acid utilizes D-glucose as the initial substrate, which is oxidized by *Erwinia herbicola* to produce a diketo-L-gluconic acid, as shown in Equation 4 (Witcoff et al. 2013). The diketointermediate undergoes a second fermentation process by *Corynebacterium*, which reduces the substrate to 2-keto-L-gluconic acid (Witcoff et al. 2013). The final step is lactonization of the sugar derivative under acidic conditions to form ascorbic acid (Witcoff et al. 2013). While the process shown in Equation 4 utilizes *Erwinia herbicola* and *Cornebacterium* to carry out the fermentation processes, these steps have been reported with a range of microorganisms including *Gluconobacter oxydans*, *Bacillus megaterium*, and *Ketogluonicenium vulgare* (USDA 1995a, Davey et al. 2000, EFSA 2013a, EFSA 2015). The microorganisms employed for the syntheses are not genetically modified (EFSA 2013a). Following fermentative transformations, ascorbic acid is isolated by acidification, ion exchange, or ultrafiltration and crystallization (EFSA 2013a, Witcoff et al. 2013, EFSA 2015). Despite the use of various microorganisms for the bulk of the synthesis, the use of acid in the final step of the process to convert the 2-keto-L-gluconic acid to ascorbic acid results in the substance's classification as "synthetic," according to the guidelines in NOP 5033-1 (NOP 2016a).


D-glucose
D-gluticol
L-sorbose
acid
acid
1) NaOMe, MeOH
2) Acid
L-Ascorbic Acid

Equation 3



Equation 4

Evaluation Question #2: 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.

Ascorbic acid is naturally produced in nearly all plants and many animal species (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, Gallie 2013). Moreover, due to the availability of synthetic sources of ascorbic acid, it has become the primary source of the substance for food handling and processing applications. Glucose is the most common substrate used in ascorbic acid synthesis (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2015). However, the production of the substance from glucose (designated as a synthetic substance by USDA

1995b), and the use of acid in the production of ascorbic acid, result in its classification as a nonagricultural substance according to the guidelines in NOP 5033-2 (NOP 2016b).

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

This report is focused on synthetic ascorbic acid due to the lack of nonsynthetic commercial sources. While ascorbic acid is naturally produced in nearly all plants and many animal species, its reactive nature makes isolation of the substance from natural sources challenging, which has resulted in all commercial ascorbic acid being synthetically derived (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, Gallie 2013). Although the substance is biosynthetically produced in both plants and animals (except for some animals, including humans), plants are the primary source of natural ascorbic acid (Davey et al. 2000). More specifically, ascorbic acid is obtained naturally in fruits and vegetables, with fruits typically being the richest sources of natural ascorbic acid. (Davey et al. 2000). Acerola (West-Indian cherry), blackcurrant, guava, and rosehip have especially high ascorbic acid contents (Davey et al. 2000). Despite the prevalence of the substance in biological systems and natural foodstuffs, all commercial sources of pure ascorbic acid are synthetically produced.

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.

The United States Food and Drug Administration (FDA) has granted ascorbic acid GRAS status for use as a chemical preservative at 21 CFR 182.3013 and §582.3013 (for animal feeds), as well as for use in nutrients and dietary supplements at §182.8013 and §582.5013 (for animal feeds).

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)).

The biological prominence of ascorbic acid has resulted in a range of roles in terms of food handling and processing, including use as a preservative (Pizzocaro et al. 1993, Frankel 1996, Vermeiren et al. 1999, Choi et al. 2002, Tapia et al. 2008, Rojas-Grau et al. 2009). Ascorbic acid is often included in food products as a preservative. Preservative applications of ascorbic acid make use of its antioxidant properties to prevent discoloration in a range of processed products, and protection from spoilage in lipid-based products (e.g., food oils) (Pizzocaro et al. 1993, Frankel 1996, Vermeiren et al. 1999, Choi et al. 2002, Tapia et al. 2008, Rojas-Grau et al. 2009).

Ascorbic acid is the most commonly used as a preservative to prevent enzymatic browning reactions that occur during processing and post-processing storage (McEvily et al. 1992, Pizzocaro et al. 1993, Tapia et al. 2008, Rojas-Grau et al. 2009). This activity is explained through a variety of mechanisms, including reactions with oxidants (e.g., free radicals and molecular oxygen) (Vermeiren et al. 1999, Davey et al. 2000). When ascorbic acid neutralizes these oxidizing species through reduction (electron transfer, shown in Equation 1), the oxidative spoilage pathways (responsible for most discoloration and changes to flavors and odors) are disrupted (Gill and McGinnes 1995, Schozen et al. 1997, Berenzon and Saguy 1998, Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009, EFSA 2015). The reducing nature of ascorbic acid deactivates polyphenol oxidase (PPO), the most common enzyme in discoloration and enzymatic browning reactions (McEvily et al. 1992, Pizzocaro et al. 1993, Tapia et al. 2008, Rojas-Grau et al. 2009). The reducing nature and oxygen-scavenging ability of ascorbic acid has also been shown to increase resistance to mold and fungal growth (Vermeiren et al. 1999).

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)).

Ascorbic acid is not intended to recreate or improve flavors and does not alter the colors or textures of food products. However, the preservative properties of ascorbic acid (discussed in greater detail in Question #5) act to prevent discoloration and degradation associated with the formation of undesirable flavors and odors (Gill and McGinnes 1995, Schozen et al. 1997, Berenzon and Saguy 1998, Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009, EFSA 2015).

Ascorbic acid is a relatively unstable compound, and its reducing nature makes it reactive to air, light, moisture (including humidity), metals (e.g., iron (Fe^{3+}) and copper (Cu^{3+})), and bases (Vermeiren et al. 1999, Davey et al. 2000, EFSA 2013b, Sigma-Aldrich 2014). This instability dramatically reduces the concentration of the natural vitamin in many processing applications (Vermeiren et al. 1999, Davey et al. 2000, EFSA 2013b). The reactive properties of ascorbic acid also make it susceptible to background reactions (part of its preservative character), and studies have shown the concentration to decrease during storage, especially in cases of neutral to high pH and in the absence of reduced temperatures (Vermeiren et al. 1999, Davey et al. 2000, EFSA 2013b, Sigma-Aldrich 2014). Moreover, these considerations are dramatically increased in terms of final food preparation (cooking). The low thermal stability, coupled with the high-water solubility of ascorbic acid results in dramatic reductions in most cooking processes, especially when extended time is required in water at elevated temperatures (e.g., boiling) (Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009). Due to the daily dietary requirements and relative instability of ascorbic acid, it is often added to processed foods (Frankel 1996, Vermeiren et al. 1999, Choi et al. 2002, Teucher et al. 2004, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015).

Evaluation Question #7: 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)).

Ascorbic acid serves to enhance the nutritional quality of food/feed to which it is added. Humans are among several animals that are unable to naturally produce ascorbic acid, making it an essential vitamin (Chatterjee 1973, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015). Since ascorbic acid is also water-soluble, it is unable to be stored in the body, and therefore must be consumed daily (Timberlake 2015). Moreover, the reducing nature of ascorbic acid results in its relatively low stability, and natural concentrations are dramatically reduced in the handling, processing, storage, and preparation of food products (Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009). The addition of ascorbic acid to certain foodstuffs is important to maintain the daily intake values required for optimum biological function (Chatterjee 1973, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015).

Evaluation Question #8: 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)).

There have been few reports of heavy metal contamination in industrially produced ascorbic acid. The European Food Safety Authority (EFSA) has reported some ascorbic acid produced via microorganism fermentation to have heavy metals present at concentrations of <20 mg/kg, and lead content of <2 mg/kg (EFSA 2013a). The reports of lead content of 2 mg/kg (parts per million (ppm)) is in excess of FDA tolerances for bottled water (5 parts per billion) (ppb)), candy (0.1 ppb), and juice (50 ppb) (FDA 2019). The low concentration (in terms of the final composition of the foodstuff) of ascorbic acid added in the handling and processing of food products makes assessing the toxicological impact of any heavy metal contamination from the substance difficult. Beyond these tolerances for finished food products, the FDA provides no additional information regarding heavy metals and other contaminants.

Evaluation Question #9: 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)).

At the time of publication of this report, the author found no published studies on the environmental persistence or impacts to biodiversity of ascorbic acid. Given the natural prevalence of the substance in plants and animals, the incorporation of ascorbic acid in the handling/processing of organic food products is unlikely to provide any significant increase to environmental levels of the substance (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, Gallie 2013). Moreover, the requirement of the substance for proper biological function in both plants and animals provides countless sources of metabolism, and the documented low toxicity of ascorbic acid make environmental contamination or harmful environmental outcomes unlikely (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, Gallie 2013).

Most commercial ascorbic acid is derived from the fermentation of glucose (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2015). Given the ubiquitous nature of glucose in biological systems, and the relatively benign conditions required in fermentation processes, the industrial production of ascorbic acid is unlikely to be a source of environmental harm.

Evaluation Question #10: 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)).

Humans are among several animals that are unable to naturally produce ascorbic acid, making it an essential vitamin (Chatterjee 1973, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015). Since ascorbic acid is also water-soluble, it is unable to be stored in the body, and therefore must be consumed daily (Timberlake 2015). The ability of ascorbic acid to act as a reducing agent makes it an important cofactor, which typically helps to promote enzymatic activity by maintaining the reduced form of iron and copper at the active site (Fe^{2+} and Cu^{+}, respectively) (Levine and Hartzell 1987, Padh 1990, Ghosh et al. 1997, Jung and Wells 1997, Davey et al. 2000). This cofactor activity has been reported for a range of enzymatic processes, although it is most widely known in the biosynthesis of the collagen protein (Holst and Frolich 1907, Svirbely and Svent-Gyorgi 1932, Svirbely and Svent-Gyorgi 1933, Davey et al. 2000). Ascorbic acid has been shown to promote the active Fe^{2+} state in iron dioxygenases that are essential for the crosslinking of the collagen helix (Padh 1990, Davey et al. 2000). In the absence of ascorbic acid, enzymes requiring a reduced state no longer function properly. In the case of collagen synthesis, ascorbic acid deficiencies manifest themselves as scurvy (Holst and Frolich 1907, Svirbely and Svent-Gyorgi 1932, Svirbely and Svent-Gyorgi 1933, Davey et al. 2000).

The reducing ability of ascorbic acid makes it an important biological antioxidant, providing protection from ROS. (Simic and Karel 1980, Frankel 1996, Halliwell 1996, Davey et al. 2000, Carocho and Ferreira 2013). In the absence of ascorbic acid or other antioxidants, ROS produce undesired modifications to biological structures such as DNA, RNA, proteins, and lipids, which may result in mutations (Lu et al. 2010, Craft et al. 2012, Caracho and Ferreira 2013, Timberlake 2015).

The earliest applications of ascorbic acid in modern medicine include treatments for scurvy, dietary supplements, and fortification to prevent the onset of the disease (Jungeblut 1937, Davey et al. 2000, EFSA 2015, Timberlake 2015, Cho et al. 2018). Since these early findings, there has been a great deal of investigation into the effects of ascorbic acid on immune responses, from cold to cancer treatments (Jungeblut 1937, Davey et al. 2000, EFSA 2015, Timberlake 2015, Cho et al. 2018).

The maintenance of reduced metallic enzymes (Fe^{2+}, Cu^{2+}) provides pro-oxidant characteristics, which have been correlated to improved immune responses (Duarte and Lunec 2005, Carocho and Ferreira 2013). In these circumstances, the reduction of Fe^{3+} to Fe^{2+} promoted by ascorbic acid results in the formation of a ROS, which can react with nearby biological systems (Davey et al. 2000, Duarte and Lunec 2005, Carocho and Ferreira 2013). The conditions that result in ROS formation are typically related to an immune response, where the ROS reacts with a pathogen such as a virus or bacteria (Davey et al. 2000, Duarte and Lunec 2005, Carocho and Ferreira 2013).

Ascorbic acid is noted as having very low human toxicity (JECFA 1981, EFSA 2013a, EFSA 2013b, EFSA 2015). Ascorbic acid is absorbed in the gastrointestinal tract; however, absorption has been noted to be proportional to ascorbic acid levels (JECFA 1981, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015). This absorption mechanism effectively limits ascorbic acid uptake to levels required for normal physiological function, while the high-water solubility of the substance results in the excretion of unabsorbed ascorbic acid, primarily in urine (Baker et al. 1966, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015).

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

Ascorbic acid is an essential dietary vitamin in humans, and therefore is not replaceable for proper physiological function (Chatterjee 1973, Davey et al. 2000, EFSA 2013a, EFSA 2013b, EFSA 2015, Timberlake 2015). Ascorbic acid is naturally produced in nearly all plants and many animals, making alternative, natural sources possible (Crawford 1982, Davey et al. 2000, EFSA 2013a, EFSA 2013b, Gallie 2013). However, the relative instability of ascorbic acid results in its degradation through typical processing, storage, and preparation protocols (Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009). Efforts to increase the abundance of natural sources of ascorbic acid include alterations to typical processing, minimal storage (or storage at reduced temperatures), and alternative preparation methods that avoid exposure to high temperatures, water, and high pH (Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009, Sigma-Aldrich 2014). The natural concentration of ascorbic acid may also be maintained by the exclusion of oxygen. Oxygen has been reported to react with ascorbic acid when combined with light or moisture and is also commonly required to produce other reactive species (e.g., free radicals) that reduce ascorbic acid levels (Vermeiren et al. 1999, Lee et al. 2003, Rojas-Grau et al. 2009, Sigma-Aldrich 2014). In some cases, these alternatives are not possible while maintaining the organoleptic properties of the food or are limited by the seasonal availability of products and established means of distribution.

There are many possible alternatives to ascorbic acid in food preservation. These include reduced temperatures, which retards enzymatic activity responsible for discoloration and other spoilage mechanisms (Timberlake 2015). Storage at reduced temperatures also slows the growth of microorganisms responsible for food degradation (Rahman 2007).

The application of organic acids (e.g. fruit juices) are traditional means of food preservation, and possible alternatives for the inclusion of ascorbic acid in processed foods (Pizzocaro et al. 1993, Frankel 1996, Vermeiren et al. 1999, Davey et al. 2000, Choi et al. 2002). Weak organic acids (e.g. citric acid and lactic acid) have been shown to inhibit food discoloration by changing the pH of the food product, deactivating browning enzymes, denaturing protein structure, and discouraging microbial growth (Timberlake 2015). The mode of action of these substances is dependent on the specific application, although membrane disruptions (lowering the intracellular pH and subsequent metabolic inhibition) are most common (Brul and Coote 1999, Vermeiren et al. 1999). The efficacy of weak organic acids in preservation has also been explained due to their ability to chelate metallic micronutrients (e.g. Ca^{2+}, Zn^{2+}), which competes with oxidative enzymes that require these ions for their activity and as nutrients for microorganisms (Brul and Coote 1999). However, the inability of these acids to provide the reducing power of ascorbic acid prevents preservative action against reactive oxidized species and limits their efficacy against viral contamination (Baert et al. 2009).

The use of salt treatments (e.g. sodium chloride (NaCl)) are another traditional means of food preservation. Like acids, salts disrupt the regulation of cellular processes, including membrane transport, by changing the natural concentration gradient (Timberlake 2015). Moreover, the use of salt inhibits the growth of microorganisms through cellular dehydration due to water migration from the cellular matrix (Timberlake 2015).

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)).

In terms of the preservative function of ascorbic acid, alternative substances include acids such as citric and lactic acid, both of which are permitted nonsynthetic substances at 7 CFR 205.605(a). As discussed in question #11, weak organic acids (e.g. citric acid and lactic acid) inhibit food discoloration by changing the pH of the food product, deactivating browning enzymes, denaturing protein structure, and discouraging microbial growth (Brul and Coote 1999, Vermeiren et al. 1999, Timberlake 2015). However, the inability of these acids to provide the reducing power of ascorbic acid prevents preservative action against reactive oxidized species and limits their efficacy against viral contamination (Baert et al. 2009).

An additional means of food preservation in handling and processing applications is the use of controlled atmospheres. In these cases, atmospheres for food packaging and storage are chosen with limited or no oxygen to retard microbial-based spoilage (Hirsch et al. 1977, Puligundla et al. 2012). Nitrogen (N_2) and carbon dioxide (CO_2) gasses are the most common atmospheres employed which result in reduced microbial metabolic rates even in the presence of oxygen based on their ability to compete with oxygen (O_2) as protein substrates (Wayne 2000, Puligundla et al. 2012). However, the use of controlled atmospheres in packaging and processing has also been known to affect the color and other organoleptic properties (sensory properties related to food. e.g. taste, smell, texture) of the foods (Hirsch et al. 1977).

Another alternative is to employ processing procedures that feature fruit juices or to fortify food products with natural fruit juices, rather than synthetically produced ascorbic acid. However, this strategy is limited by the relative instability of ascorbic acid. Moreover, the presence of additional substances present in fruit juices may result in undesired changes to the organoleptic properties of the processed foods.

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

Ascorbic acid is present in agricultural products, and the synthetic form of the substance could be replaced with natural, organic, ascorbic acid. This is possible with the incorporation of foods high in ascorbic acid (fruits and vegetables) as ingredients in the final processed food product (Davey et al. 2000). Another alternative is to employ processing procedures that feature fruit juices or to fortify food products with natural fruit juices, rather than synthetically produced ascorbic acid.

Report Authorship

The following individuals were involved in research, data collection, writing, editing, and/or final approval of this report:

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All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11—Preventing Personal Conflicts of Interest for Contractor Employees Performing Acquisition Functions.

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