Triethyl Citrate
Handling/Processing

Triethyl Citrate

Chemical Names:
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, triethyl ester; Citric acid, triethyl ester; Citric acid derivative, ethyl ester; Ethyl citrate; Triethyl 2-hydroxy-1,2,3-propanetricarboxylate; β-hydroxytricarballylic acid

Other Names:
Ethyl citrate; TEC

Trade Names:
Citroflex 2; CITROFOL®AI, Eudraflex; Hydragen CAT

CAS Numbers:
77-93-0

Other Codes:
E No. 1505; INS No. 1519; EINECS No. 201-070-7; Council of Europe No. 11762; FEMA Number 3083; Flavis number 9.512; MDL number MFCD00009201; PubChem Substance ID 24901458; CID No. 6506

Summary of Petitioned Use

Triethyl citrate is being petitioned for addition to section 205.605 as a food additive for stabilizing foams, specifically as a whipping enhancer for organic egg whites during processing.

Characterization of Petitioned Substance

Composition of the Substance:
Triethyl citrate (TEC) is the triethyl ester of citric acid. It is formed by the esterification[1] of citric acid where ethyl groups from the ethanol replace all three of the carboxyl groups in the citric acid. (Rowe 2009; Silberberg 1996). Its chemical structure is shown in Figure 1.

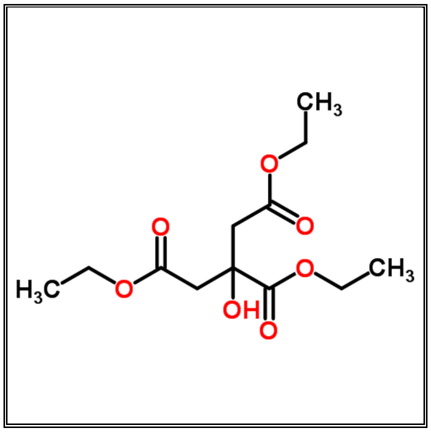

Figure 1: Triethyl citrate
(Royal Society of Chemistry 2014)

Molecular formula: $C_{12}H_{20}O_7$
Molecular weight: 276.283

[1] Esterification is the chemical conversion of an acid into an ester through combination with an alcohol and removal of water (RCOOH + ROH -> RCOOR + H_2O).

Source or Origin of the Substance:

Triethyl citrate occurs naturally as a component of plant and animal tissues (JECFA 1973). Commercial sources of TEC are produced from the reaction of citric acid and ethyl alcohol (ethanol), both of which are fermentation products from the microbial digestion of a carbon substrate. Citric acid is also produced from the fungal fermentation of glucose (Kolah, Asthana, et al. 2007).

Properties of the Substance:

The physical and chemical properties of triethyl citrate are given in Table 1.

Table 1: Properties of triethyl citrate (International Programme on Chemical Safety 1999)

Property	Characteristic / Value
Form	Oily Liquid
Color	Nearly colorless
Odor	None
Boiling Point	294ºC
Melting Point	-55ºC
Solubility in Water	Moderate
Vapor Pressure	0.3 Pa at 25 ºC
Flash Point	151ºC

Specific Uses of the Substance:

Triethyl citrate is used in a broad range of applications, from food processing to use as a plasticizer in numerous different fields. It was petitioned for use is as a whipping agent for egg whites and other foams. It is also used as a flavoring agent, a solvent, and in food coatings, food contact materials and food packaging (Kolah, Asthana, et al. 2007).

For the petitioned purpose, TEC is added to egg whites to shorten the whipping time required to reach the desired density. One patent reported that the addition of 0.03% TEC to liquid egg white reduced the whip time by 55% (Ziegler and Buehler 1965). It also stabilizes egg white foams (McGee 2004) and increases egg whites' tolerance to overbeating (Kothe 1953). TEC is most often used in pasteurized liquid egg whites, dried egg whites (Triller and Hüttl 2014) and frozen egg white products (Johnson and Zabik 1981b).

TEC may enhance antimicrobial activity (Monfort, et al. 2011). Addition of TEC improves the texture of pasta when microwaved, facilitates low temperature drying without cracking, and reduces cooking moisture losses (Chawan, Merritt and Hargrove, 1991).

TEC is also used as a flavoring agent, a solvent, and in food coatings, food contact materials and food packaging (Kolah, Asthana, et al. 2007). In general, it serves as a carrier solvent and a sequestrant (JEFCA 1973). The Codex Alimentarius General Standard for Food Additives (GSFA) Provisions for TEC include use in dried and/or heat coagulated egg products, liquid egg products, and in water-based flavored drinks, including "sport," "energy," or "electrolyte" drinks (FAO/WHO 2014).

Non-food uses of TEC are as a plasticizer, an agglutinant, and a sequestrant (Clark and Macquarrie 2008). Applications include in toys, pharmaceutical and medical products, cigarettes, printing ink coatings, cosmetics, perfumes and lacquers. It is known as an environmentally friendly, non-toxic plasticizer that can replace the highly persistent plasticizers such as phthalates (Triller and Hüttl 2014). It is also used as a fragrance carrier (Rowe 2009), an antioxidant, and is an active ingredient in deodorant products.

Approved Legal Uses of the Substance:

Triethyl citrate is listed at 21 CFR 184.1911 as a Direct Food Substance Affirmed as Generally Recognized As Safe (GRAS). The ingredient may be used in food with no limitation other than current good manufacturing practices. The section of 21 CFR refers to specific uses as a flavoring agent, a solvent and vehicle, and as a surface-active agent.

Triethyl citrate is listed in the Codex Alimentarius General Standard for Food Additives as a food additive under the following functional classes: Carrier, Emulsifier, Sequestrant, and Stabilizer in the food category: Liquid egg products, with a maximum level of 2500mg/kg. It is also listed in the "Dried and/or heat coagulated egg products" category (Max Level 2500 mg/kg), and in water-based flavored drinks, including "sport," "energy," or "electrolyte" drinks and the "particulated" drinks category (Max Level 200 mg/kg) [Codex Stan 192-1995].

While the GRAS listing for TEC covers its use as a flavor, the FDA definition of natural flavor does not support the designation of TEC as natural for this purpose, according to 21 CFR 101.22 (a)(3):

> The term *natural flavor* or *natural flavoring* means the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in food is flavoring rather than nutritional. Natural flavors include the natural essence or extractives obtained from plants listed in 182.10, 182.20, 182.40, and 182.50 and part 184 of this chapter, and the substances listed in 172.510 of this chapter.

Triethyl citrate is also listed at 21 CFR 175.300 as an Indirect Food Additive: Adhesives and Components of Coatings, Subpart C – Substances for Use as Components of Coatings. Its specific listing is under Resinous and polymeric coatings.

The petition for this substance lists it as "Triethyl Citrate (TEC), Natural (Organic Compliant)" and states as a current application "Flavor – Organic." Section 205.605(a) of the NOP Rule allows non-organic, non-agricultural natural flavors in organic foods provided that they are from "nonsynthetic sources only and must not be produced using synthetic solvents and carrier systems or any artificial preservative." Because TEC is only commercially available in synthetic form, it does not appear to meet the NOP requirements for use as a flavor in organic food products. The esterification process described in the petition and this report involves a chemical reaction of ethanol and citric acid, rendering it a synthetic substance.

Action of the Substance:

To understand the action of TEC as a whipping agent, one must understand how foaming occurs with egg whites. Egg white (albumen) is one of the best food foaming agents in food applications (Saint-James, Durian and Weitz 2012). Meyer and Potter (1975) explain that foaming occurs as liquid materials concentrate at the surface and form a layer around an air inclusion. When egg whites are whipped, the proteins unfold due to both the pulling force of the whisk, and the air that becomes incorporated with the liquid whites (McGee 2004). The denatured proteins form an electron-dense layer at the interface of the water in the egg whites and the air. Different albumen (egg white) proteins, both alone and in combination, function differently in foaming and stabilizing the foam (Johnson and Zabik 1981b).

Globulins, ovotransferrin (conalbumin) and ovomucin are the principal egg white proteins that denature and bond together at the air-water interface known as foam (McGee 2004). Other proteins such as lysozyme, ovalbumin, and ovomucoid are said to have little to no foamability (Meyer and Potter 1975; Johnson and Zabik, 1981b). Interactions between proteins are also important. A concentration of cross-linked proteins from an ovomucin-lysozyme complex has been reported to improve foam appearance

(Johnson and Zabik 1981a), but is also more subject to denaturation by heat—such as the temperatures that occur in pasteurization—than either of the proteins alone (Garibaldi, et al. 1968). Ovomucin has been cited as the protein responsible for foam stability (Garibaldi, et al. 1968), and ovalbumin reinforces the foam structure when heated, such as when baking a meringue or soufflé (McGee 2004).

Triethyl citrate performs several functions as a whipping agent. Meyer and Potter (1975) suggested that it denatures proteins, thus increasing the solid matter that can coagulate at the surface area of air pockets, which increases foamability. As proteins unfold, when they are denatured the surface tension decreases to further facilitate foam formation. One study concluded that the addition of TEC to pasteurized egg whites before whipping greatly increased foam stability and improved luster. Its authors suggested that TEC alters the egg whites' physical properties, such as surficial viscosity, such that the foam produced is less susceptible to mechanical damage (Garibaldi, et al. 1968). McGee (2004) explains that the proteins responsible for foaming in egg whites can also destabilize the foam. Too many bonds between the denatured proteins can cause the structure to begin to collapse. Some of these are sulfur bonds that form when a protein's S-H bonds break and form an S-S bond with another protein. The donation of H^+ ions from TEC or another acid diminishes this phenomenon, making the foam more stable.

The foaming potential of egg whites is altered depending on pasteurization parameters and drying processes, and is also affected by temperature (Penfield and Campbell 1990). TEC is commonly added as a whipping agent to dried, frozen or refrigerated egg white products (American Egg Board 2013).

Citrates including TEC can also function as sequestrants or chelating agents in foods that are sensitive to oxidation, such as fats and oils. Trace metals serve as catalysts for oxidation in these substances, causing rancidity and off-flavors. When TEC complexes these metals, their catalytic effect on oxidation is inactivated or reduced (Furia 1973).

Combinations of the Substance:

Triethyl citrate is commonly used as a stand-alone food additive and does not require formulation with other substances in order to be used as a whipping agent. A review of commercially available conventional liquid and dried egg white products suggests that the most common forms are egg whites without additives, followed by egg whites with triethyl citrate as a whipping agent and egg whites with guar gum and triethyl citrate added as whipping agents (Ballas Egg Products Corp. 2013; Michael Foods, Inc. 2013).

However, there are formulated food additives that use triethyl citrate in combination with other ingredients to aid in whipping or to stabilize foam. Meyer and Potter (1975) reported on the use of triethyl citrate plus trisodium citrate, and found that TEC improved foamability via increased denaturization of ovalbumin, while trisodium citrate increased foam stability by crosslinking proteins (Meyer and Potter 1975). Sodium citrate is used as an additional whipping agent in combination with triethyl citrate in at least one commercial egg white product which also contains xanthan gum (National Food Corporation 2009). Xanthan gum and other polysaccharides such as guar gum and carrageenan may be added to egg whites to increase the stability of products with high-sugar content. These polysaccharides interact with the proteins in albumen via hydrophobic interactions, hydrogen bonding and electrostatic interactions, thereby increasing viscosity and lowering surface tension (Miquelim, Lannes and Mezzenga 2010). These additives can function without TEC and are discussed further under Question 11.

One patent claimed that TEC used in combination with gelatin, also used as a whipping agent, improved the gelatin's whipping properties in marshmallows and stable foam food preparations (Conrad and Stiles 1954). Monosodium phosphate and sodium acid pyrophosphate are other additives seen in combination with TEC in egg whites intended for use in high-sugar products such as marshmallow foams, cream fillings and candies (Ballas Egg Products Corp. 2013).

Triethyl citrate may be used as a sequestrant in combination with an antioxidant, often with synergistic effects. Sequestrants reduce or eliminate oxidation caused by trace metals, while antioxidants hinder oxidation by chain termination. Examples of antioxidants that are used in combination with TEC include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA), propyl gallate, ascorbic and isoascorbic acids, phopholipods and various thiopropionates (Furia 1973). Most, but not all of these are synthetic. None appear on the National List at 7 CFR 205.605.

Status

Historic Use:

Triethyl citrate was first patented for use as a whipping aid in eggs in the early 1950s (Kothe 1953), when it was among a number of other egg white foam-improving additives identified by food scientists (Meyer and Potter 1975). Much of the literature surrounding the use of TEC as a whipping aid was published in the 1960s and '70s. While the substance has been in use since that time, there are few recent studies on its role as a food additive.

Organic Foods Production Act, USDA Final Rule:

Triethyl citrate does not appear in the Organic Foods Production Act of 1990 and does not appear anywhere on the USDA National Organic Program's National List.

International

Canada - Canadian General Standards Board Permitted Substances List –
http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/internet/bio-org/index-eng.html
Triethyl citrate is not a permitted substance at CAN/CGSB-32.311-2006. It does not appear in Table 6.3 "Non-organic Ingredients Classified as Food Additives."

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999) - ftp://ftp.fao.org/docrep/fao/005/Y2772e/Y2772e.pdf
Triethyl citrate does not appear in the CODEX Alimentarius Commission Guidelines for the Production, Processing, Labelling, and Marketing of Organically Produced Food.

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008
http://www.organic-world.net/news-eu-regulation.html
http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_189/l_18920070720en00010023.pdf
Triethyl citrate is not permitted in the European Union as a food additive in organic processed foods. It does not appear in EN 2092/2008 Annex VIII Section A – Food Additives, Including Carriers, or Section B – Processing Aids and Other Products, Which May Be Used for Processing of Ingredients of Agricultural Origin from Organic Production.

Japan Agricultural Standard (JAS) for Organic Production—
http://www.ams.usda.gov/nop/NOP/TradeIssues/JAS.html
Triethyl citrate is not listed in the Japanese Agricultural Standard for Organic Processed Foods (Notification No. 1606 of the Ministry of Agriculture, Forestry and Fisheries of October 27, 2005)

International Federation of Organic Agriculture Movements (IFOAM) –
http://www.ifoam.org/standard/norms/cover.html
Triethyl citrate is not permitted by the IFOAM Standard as a food additive in organic processed foods. It does not appear in Appendix 4 – Table 1: List of Approved Additives and Processing/Post-Harvest Handling Aids.

Evaluation Questions for Substances to be used in Organic Handling

<u>Evaluation Question #1:</u> 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)).

Triethyl citrate is made by reacting citric acid with ethanol in the presence of a catalyst to esterify the citric acid, first to monoethyl citrate (MEC), then to diethyl citrate (DEC), and finally to TEC as the end product (Kolah, Asthana, et al. 2007). See Figure 2. The precursors citric acid and ethanol are both commonly nonsynthetic and react chemically to form TEC.

Citric acid is primarily produced through fermentation of carbohydrate substrates by the fungus *Aspergillus niger*, and recovered by precipitation, extraction or adsorption (Soccol, et al. 2006). Ethanol is similarly produced by the fermentation of carbohydrate substrates, typically agricultural, by yeasts such as *Saccaromyces cereviciae*, and recovered by distillation.

TEC is commonly produced by the reaction of the two substances, citric acid and ethanol, when heated and in the presence of a catalyst such as sulfuric acid or an ion-exchange resin. The reaction can also be self-catalyzed by citric acid. Citric acid and ethanol react relatively quickly to form mono-ethyl citrate (MEC), while diethyl citrate (DEC) forms slightly more slowly, and production of triethyl citrate is much slower and harder to achieve. Because the esterification of citric acid is slow and limited by the equilibrium of the forward and reverse reactions, multiple stages must be used. Alternatively, triethyl citrate can be removed as the reaction is occurring in a process called reactive distillation. Using this method, Kolah, et al. (2007) found that the rate of conversion of citric acid to TEC was increased at higher temperature (120ºC), higher ethanol : citric acid molar ratio (15:1), and with catalyst loading (5 wt %of the reaction solution). They report that increasing these parameters in order to produce more TEC is limited by the increased formation of diethyl ether (DEE) through the dehydration of two ethanol molecules at higher temperatures and catalyst levels. A subsequent study by the same authors explored optimal conditions for producing TEC by reactive distillation, and obtained finished TEC product with a 98.5 wt % purity and the main by-product being diethyl citrate (Kolah, Asthana, et al. 2008).

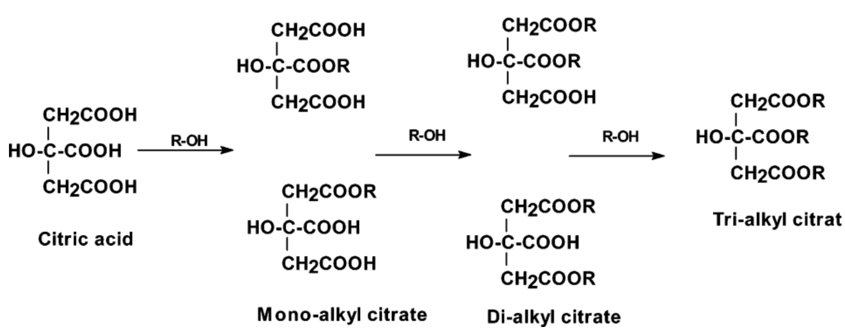

Figure 2: Esterification of Citric Acid
(Kolah, Asthana, et al., Reaction Kinetics of the Catalytic Esterification of Citric Acid with Ethanol 2007)

Another method for reacting citric acid and ethanol to produce triethyl citrate is described by Frappier et al. (2002). In their proposed method, ethanol is added to citric acid that is still in the fermentation broth state[2]. This produces a heterogeneous organic reacted mixture in an organic phase containing the citrate esters, including triethyl citrate, and an aqueous phase containing water soluble impurities, which are then separated out by distillation (Frappier, et al. 2002). The drawback of this method for application as a food additive is that it does not isolate TEC from MEC and DEC; however it is less energy intensive than

[2] The fermentation broth is described as containing approximately 10% or more citric acid by weight as well as salts, carbohydrates, proteins amino acids and other materials. It is partially purified by the removal of cationic impurities prior to esterification.

reactive distillation using purified citric acid. Thus, the intended application of this method is the economical production of citrate esters for use as industrial plasticizers.

The petition for the addition of TEC to the National List provides a manufacturing process in which citric acid and ethanol are combined to cause esterification. This is followed by evaporation to remove ethanol and water, and the material then undergoes purification using unidentified chemical agents. The product undergoes a second purification step of distillation, after which it is stored and filtered prior to packaging.

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.

Triethyl citrate is formulated by the chemical process of esterification. The reagents—citric acid and ethanol—both come from the naturally occurring biological process of fermentation of agricultural materials, as discussed earlier. While ethanol is considered agricultural, citric acid is considered nonagricultural and nonsynthetic. TEC is the triethyl ester of citric acid. It is formed by the esterification of citric acid where ethyl groups from the ethanol replace all three of the carboxyl groups in the citric acid. (Rowe 2009; Silberberg 1996). The formation of TEC occurs when oxygen atoms in the ethanol's –OH groups are attracted to the carbon atoms in each of the three carboxyl groups of the citric acid and bond to form the ester. The OH^- from the citric acid combines with the H^+ from the ethanol to form water as a by-product (Silberberg 1996).

One study reported the extraction of triethyl citrate from the brown seaweed, *Ishige okamurae*, along with pyroglutamic acid (PGA) and di-n-octylphthalate (DNOP) (Cho, et al. 2005). In the study, the seaweed powder was extracted with methanol-water and fractionated by polarity; the moderately polar extract was further fractionated and the active fractions separated by reverse-phase high-performance liquid chromatography (HPLC). This type of fractionation is considered a physical extraction process rather than chemical. The study's purpose was to understand the extract's function as an algal inhibiting or antifouling agent and was not explored as a method for commercial extraction.

There is also a patent application filed that claims that it is possible to produce TEC from tobacco through fermentation and distillation (Dube and Coleman 2011). The patent is pending and no TEC is currently known to be commercially available from this source.

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

There are no known commercial sources of non-synthetic or natural triethyl citrate. Numerous plants and animals are non-commercial sources, including brown seaweed and tobacco.

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.

Triethyl citrate is listed as GRAS at 21 CFR 184.1911. The GRAS listing states that the ingredient may be used in food with no limitation other than current good manufacturing practices. The affirmation of this ingredient as GRAS as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as a flavoring agent as defined in 170.3(o)(12) of this chapter; a solvent and vehicle as defined in 170.3(o)(27) of this chapter; and a surface-active agent as defined in 170.3(o)(29) of this chapter.
(2) The ingredient is used in foods at levels not to exceed current good manufacturing practice.

(d) Prior sanctions for this ingredient different from the uses established in this section, or different from those set forth in part 181 of this chapter, do not exist or have been waived.

Section 21 CFR 170.3(o)(12) defines flavoring agents and adjuvants as: substances added to impart of help impart a taste or aroma in food. Section 21 CFR 170.3(o)(27) defines solvents and vehicles as: substances used to extract or dissolve another substance. And 21 CFR 170.3(o)(29) defines surface active agents as: substances used to modify surface properties of liquid food components for a variety of effects, other than emulsifiers, but including solubilizing agents, dispersants, detergents, wetting agents, rehydration enhancers, whipping agents, foaming agents, and de-foaming agents, etc.

The petitioned use for this substance falls under 21 CFR 170.3(o)(29) and is covered by the GRAS Listing.

<u>Evaluation Question #5:</u> 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)).

A chemical food preservative is defined under FDA regulations at 21 CFR 101.22(a) (5) as "any chemical that, when added to food, tends to prevent or retard deterioration thereof, but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties"(FDA 2013). The addition of triethyl citrate to egg whites as a whipping agent functions both to aid in the foaming process and to help stabilize the whipped foam. Stabilization of the foam could be considered a preservative function, although it prevents deterioration not of the egg white itself but of the structure achieved by the whipping action. The mechanism by which TEC stabilizes foam is discussed above under Action of the Substance.

As a sequestrant, TEC helps establish, maintain and enhance the integrity of the food product in which it is used (Furia 1973).

<u>Evaluation Question #6:</u> 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)).

The main reason that TEC is added to egg whites is to recreate textures and related properties that are lost during pasteurization. According to the Food Safety and Inspection Services (FSIS; 2013), most egg products other than fresh shell eggs— including liquid and powder egg whites—are pasteurized in the U.S., as required by the 1970 Egg Products Inspection Act. Pasteurization of egg whites at 53°C causes denaturation of important proteins essential to the foaming capacity which results in reduced quality and volume of the foam itself and the resulting angel food cakes and other baked goods. TEC increases the denaturation temperature and improves the foaming properties of egg white when added after pasteurization (Lomakina and Mikova 2006). Specifically, Garibaldi, et al. (1968) found that the addition of TEC to pasteurized egg whites before whipping greatly increases foam stability and improves luster. TEC also alters egg whites' physical properties, such as surficial viscosity, so that the foam produced is less susceptible to mechanical damage (Garibaldi, et al. 1968). See "Action of Substance" for more information about how TEC affects the texture of egg whites.

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

There is no literature to suggest that the addition of TEC to egg whites has any effect or potential effect on the nutritional quality of the egg whites. One study (Monfort, et al. 2011) suggested that the addition of TEC during pasteurization by pulsed electric fields and heat contributes to a reduction of *Salmonella spp*. to acceptable levels, thereby helping to preserve egg white foaming properties.

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

The FDA stipulates that food grade triethyl citrate cannot contain more than 3 ppm arsenic and 10 ppm lead (FDA, 1977). The Food Chemical Codex (FCC; 2012) monograph of TEC stipulates that it cannot contain more than 10 ppm of lead. A review of various specification sheets indicates that major commercial sources of TEC meet the requirements set by the FDA and FCC (Acros Organics 2009; Sigma-Aldrich 2014; Vertellus 2014).

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

TEC is considered an "environmentally friendly" plasticizer and is utilized in biodegradable plastic applications (Park, et al. 2004). In several studies, it has been found to be present in waterways and thought to be introduced through sewage and wastewater discharges (Stackelberg, et al. 2007; Dsikowitzky, et al. 2004). TEC was found to be ubiquitous in both the Lippe River's (Germany) mouth and source, as well as its tributaries and wastewater effluents (Stackelberg, et al. 2007). Dsikowitzky, et al. (2004) similarly found TEC in drinking water samples at a heavily populated, highly urbanized drinking water treatment plant in the U.S. Both the untreated water and the finished water had some TEC present (0.085µg/L and 0.013µg/L respectively), demonstrating that TEC is not completely broken down by the typical water treatment process. Neither of these studies made any suppositions of the environmental effects of such TEC content in water.

TEC is favored as a plasticizer in biodegradable plastics over other plasticizers such as phthalates due to its low toxicity, relatively rapid biodegradability, and its use as a food additive and pharmaceutical excipient (Guiot, Ryan and Kennedy 1998). Citrate esters degrade more rapidly in soil than cellulose (paper) (Kouloungis 1996 cited in Guiot, Ryan and Kennedy, 1998).

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

In 1977, the FDA concluded that "there is no evidence in the available information on citric acid…and triethyl citrate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when used at levels that are now current or that might reasonably be expected in the future." According to a report by the Joint FAO/WHO Expert Committee on Food Additives (1999), despite a lack of absorption and metabolism studies, TEC is expected to rapidly metabolize in the body and liberate the citrate ion, which would then be processed via the usual biochemical pathways. The committee found that the LD_{50} for rats and cats, respectively, was 8,000 and 4,000 mg/kg of body weight. They estimated that the acceptable daily intake (ADI) for humans is between 1-10 mg/kg of body weight[3]. TEC tested negative as a mutagen (Litton Bionetics 1976 cited by the Cosmetic Ingredient Review Expert Panel, 2012).

Triethyl citrate is also used in cosmetics and has been studied for its dermal toxicity (Fiume, et al. 2014). The authors found that TEC at concentrations up to 100% was not a skin irritant in guinea pigs and rabbits, nor was it found to be an irritant for humans when 0.4 mL was applied with an adhesive square on the arm. When applied to the shaved back of guinea pigs, TEC resulted in a 12-20 minute insensitivity to continued pricking of the area. TEC inhibited the transdermal absorption of a synthetic prostaglandin, viprostol, through the skin of male hypertensive rats. A 33.3% solution of TEC applied to rabbit eyes produced irritation (Cosmetic Ingredient Review Expert Panel 2012). The Cosmetic Ingredient Review Expert Panel concluded that the alkyl esters of citric acid (including TEC) are safe to use in present cosmetic practice and concentrations.

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

[3] LD_{50} represents the individual "lethal dose" required to kill 50% of a population of test animals.

Whipping aids are considered to be optional ingredients in liquid and frozen egg whites (Bergquist 2007). The primary alternative practice to using triethyl citrate in high-whip egg white production is to omit it. Baked goods and other products with egg whites as ingredients can be made without whipping aids, or with alternative substances at the whipping stage to enhance the whipping functionality of the egg whites. Various alternatives are available for use at the stage of whipping the egg white, rather than as additives for packaging and selling ready-made, high-whip egg whites. See questions 12 and 13 below for a comparison of alternative substances that are currently permitted as ingredients in organic foods that may be used at the time of whipping to enhance foaming properties of egg whites.

Numerous culinary methods to improve egg white foam stability have been discovered by chefs over the years. Three basic approaches to increase the protein stability include: (1) whipping in a copper bowl; (2) mild heat treatment of egg white solution, and (3) the addition of simple and complex sugars and / or acids (McGee 2004; Foegeding, Luck and Davis 2006).

One alternative known to chefs since the eighteenth century is the use of copper bowls for whipping egg whites. Foam stability is enhanced due to a copper-conalbumin complex that is denaturation-resistant, and also possibly because other egg proteins have sulfhydryl groups altered during the whipping process (Luck and Foegeding 2008). Luck and Foegeding (2008) found that egg white proteins whipped in a copper bowl or in the presence of 1 mM copper sulfate showed significantly improved foam stability. Cotterill, et al. (1992) reported similar results in heat-spray dried egg whites, where copper ions added at various levels consistently increased foam firmness and stability. Drainage from whipped egg white proteins was reduced when copper ions were added to egg white powders (Sagis, et al., 2001). Figure 3 compares drainage for pure egg whites with and without copper added. Sagis, et al. (2001), however, found that adding copper had no effect on egg foam stability; they explained that this is most likely due to partial denaturation during the drying and heat treatment of the egg powder.

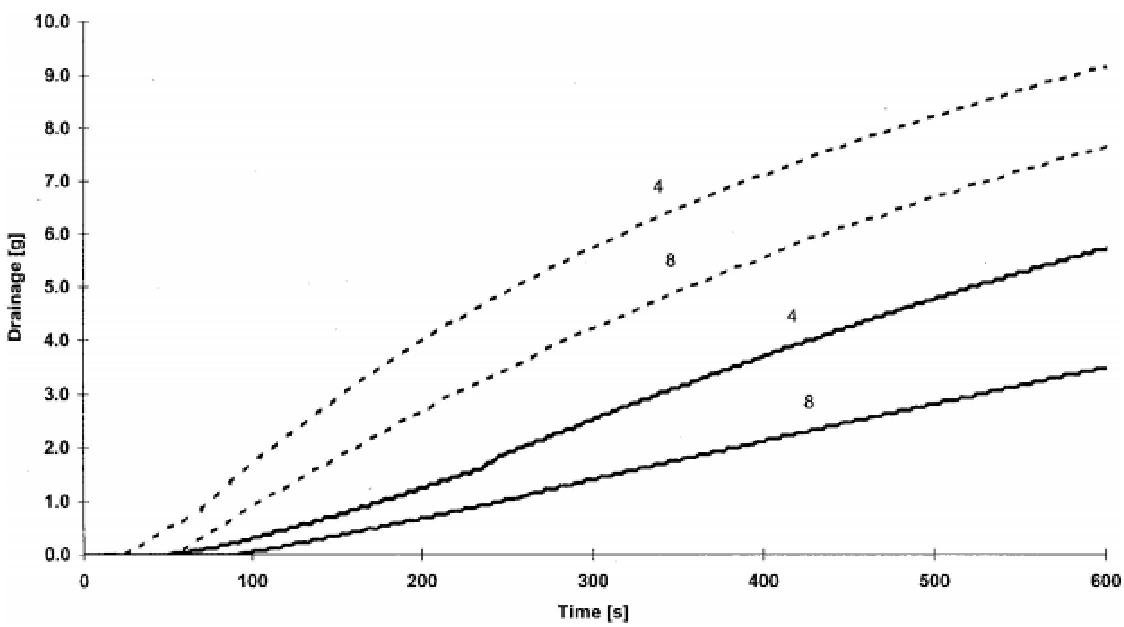

Figure 3: Drainage cure for pure egg white beating time 4 or 8 minutes, at blender setting of 660 rotations per minute (rpm). Dashed curves are the samples without copper ions, solid curves are the samples with copper ions. Labels indicate beating time. (Sagis, et al. 2001)

Another alternative practice is to reduce the effects of pasteurization on foaming properties by treating the egg whites with hydrogen peroxide during processing. Specifically, egg whites are pH adjusted with citric acid, treated with 0.1-0.8% hydrogen peroxide by weight, and then pH adjusted back to 7.5 either prior to or after freezing. This procedure results in a cake volume in the range of 38% above control cakes containing egg whites with no treatment (Leon and Strandine 1968). OvoPro, an egg processing equipment manufacturer, currently provides a high-whip egg white pasteurization kit that injects accurate amounts of hydrogen peroxide into egg whites in order to maintain high quality liquid or frozen egg white (OvoPro 2014). Section 21 CFR §160.145 permits hydrogen peroxide in the desugarization and sterilization of egg whites (FDA 2014). Although hydrogen peroxide is permitted for egg white sterilization by the FDA, Muriana (1997) found that its use in addition to heat treatment is not sufficient to render the egg white free from *L monocytogenes*. This has implications especially for egg whites that are only refrigerated rather than frozen.

Treating egg whites with papain enzymes has also been found to dramatically affect the foaming capacity, and increased amounts of papain produced higher foaming capacity (Lee and Chen 2002). The same study also found that angel food cake volume increased with papain treated egg whites. Again, the more papain used, the higher the cake volume of the egg whites.

The form of the egg white should also be considered when assessing alternatives for maintaining high foaming functionality. Franks, Zabik and Funk (1969) studied the functional properties of various forms of egg whites in angel food cakes. Cakes with foam-spray-dried albumen had greater volume than cakes with frozen and freeze-dried albumen, while cakes with spray-dried albumen had the lowest volume. Tenderness and moistness were also affected by albumen form; cakes with freeze-dried albumen scored lower in both characteristics than cakes made with frozen, foam-spray-dried and spray-dried albumens.

Rather than whipping and beating, foam can be formed in egg whites by direct aeration. Comparison of a bubbling apparatus with whipping devices showed that the bubbling method had more consistent and reproducible results (Baniel, Fains and Popineau 1997). The two actions can be combined to shorten the time required for foam formation as well as produce a more uniform texture (Gross 1964). It is not known whether there are any vendors of bubblers or aerators suitable for commercial scale processing.

Recipes often call for raising the temperature of liquid eggs to room temperature or above before beating. Mild heat will enable proteins to form soluble aggregates based on covalent intermolecular linking (Foegeding, Luck and Davis 2006). A little heat will improve foaming, but too much will decrease foaming properties. Because the pasteurization step has an adverse effect on the properties of eggs, non-thermal and low-impact methods for the reduction of food borne pathogens are being studied. One promising method is non-thermal High Pressure Processing (HPP). Eggs that were treated with HPP achieved comparable levels of *Salmonella* and *Listeria* reduction, and had higher foam stability than pasteurized eggs (Hoppe 2010). Another method is the use of high frequency radio waves (Hamid-Samimi, Swartzel and Ball 2002). Both are still experimental and are not commercially available methods to prevent pathogens in liquid egg products.

The use of sugars is further discussed under Question #13.

<u>Evaluation Question #12:</u> 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)).

The main substance used during the whipping stage to enhance egg white foaming characteristics is cream of tartar (potassium acid tartrate), which currently appears in §205.605(b) as a synthetic substance permitted as an ingredient in organic foods. Other nonsynthetic substances such as xanthan gum, guar gum and carageenan are of minor importance and have been minimally studied for effectiveness. Additionally, sugar and salt have been studied for their effects on foaming characteristics (Raikos, Campbell and Euston 2007). See question 13 for a complete discussion of this study.

Most angel food cake and merengue recipes call for cream of tartar to be added before or during the egg white whipping stage (Brown 2014; Martha Stewart 2014; allrecipes.com 2014; McGee 2004). Cream of tartar is known to lower the pH of the egg whites and increase foam stability. Changes in pH alter the charge and form of proteins, and the addition of acids such as cream of tartar lowers the pH close to the isoelectric point of the foaming proteins in the egg white. Near isolectric points, proteins pack closer at the air/liquid interface in the foam and viscosity increases (Oldham, McComber and Cox 2000). Despite its clear importance in altering the foaming characteristics of egg whites, cream of tartar has not been widely studied since the 1950's for its effects on foam. However, Oldham, McComber and Cox (2000) recently did compare the effects of different levels of cream of tartar on angel food cake quality, and found that increasing cream of tartar levels (to a point) led to increased cake volume, crumb whiteness and tenderness. The authors noted that angel food cakes made with 2.5g of cream of tartar (1/4 tsp per egg white) were the best in quality, as measured by cake volume, crumb whiteness, and tenderness.

Miquelim, Lannes and Mezzenga (2010) studied the effects of the polysaccharides xanthan gum, guar gum and carrageenan on foam stability at different pH levels. They found that long-term foam stability is correlated with the air-water interfacial properties facilitated by the protein-polysaccharide complexes. Carageenan was found to be the most effective additive at a pH of 3-4 for maintaining foam volume over 0-30 hours. All of these substances appear on the National List at §205.605(a) and (b) or §205.606 and are permitted as ingredients in organic foods. Figure 4 compares the different polysaccharide additives for foam volume effects under different pH conditions.

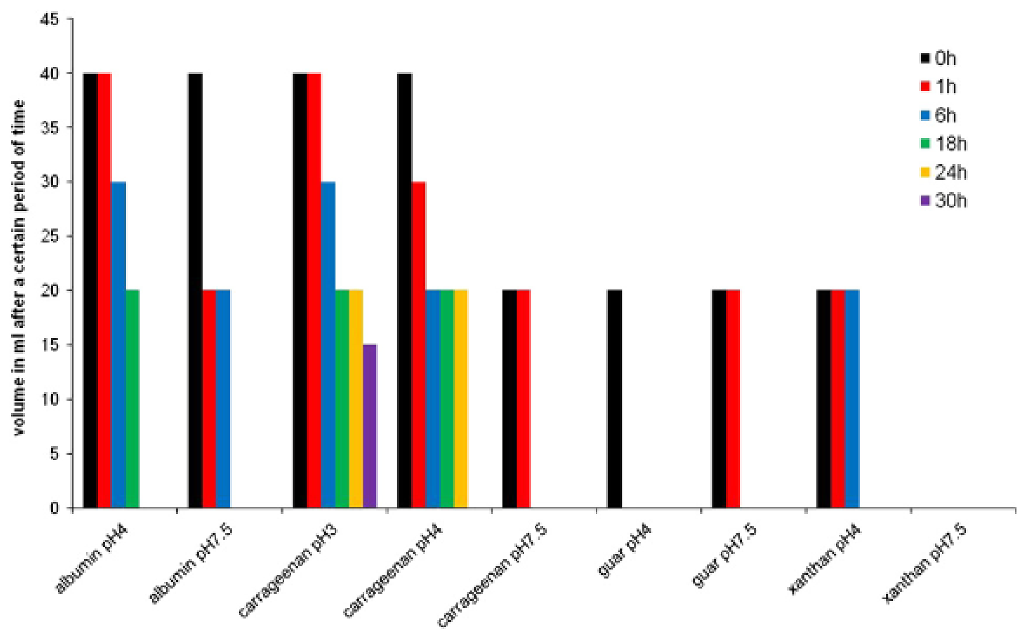

Figure 4: Foam stability versus time for different albumin/polysaccharide solutions at pHs 7.5, 4 and 3 (Miquelim, Lannes and Mezzenga 2010).

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

Sugar appears to be the longest studied and best understood agricultural product to improve the foaming characteristics of heat treated egg whites (Beilinsson 1929; Ball, Hardt and Duddles 1943; Slosberg, et al. 1948; McGee 2004) in all types of baked goods including angel food cakes and merengues. Raikos, Campbell and Euston (2007) indicate that various levels of sugar (sucrose) and salt (sodium chloride) either individually or in combination can enhance the foaming characteristics of egg albumen. Specifically, "increasing NaCl concentration, heating temperature, and whipping time enhances foam formation, whereas increasing the amount of sucrose confers foam stability for prolonged periods of whipping." Certified organic sugar is widely available across the marketplace. Salt, like water, is a non-organic non-agricultural ingredient that can be used in an organic processed food product.

Question 12 discussed the effects of guar gum on egg white foaming properties. Guar gum is agricultural and appears in section 205.606 as a nonorganic ingredient permitted in organic products when not commercially available in organic form. There are currently six sources of organic guar gum (NOP 2013).

One patent (Shaffer 1956) for treating liquid egg whites for improved properties in cake baking claims that glycerin maintains whipping properties of fermented, spray-dried egg whites. The patent claims that, in addition to a specific treatment of egg whites that includes fermentation and pH adjustments with alkaline and acid materials (such as lactic acid and sodium carbonate), glycerin is added from 4-17% by weight. While glycerin currently is considered a synthetic substance permitted as an ingredient in organic foods, there is also certified organic glycerin, with 25 sources currently available on the market (NOP 2013). While the use of glyerin as an additive for enhancing whipping properties of egg whites has been occasionally cited in the scientific literature (Beilinsson 1929; Cunningham, et al. 1965), there is no evidence that glycerin has been used commercially as an alternative to triethyl citrate to stabilize egg whites.

References

Acros Organics. "Triethyl citrate Material Safety Data Sheet." *Acros Organics.* 7 20, 2009. (accessed 8 15, 2014).

allrecipes.com. "Angel Food Cake I." *allrecipes.* 2014. (accessed August 14, 2014).

American Egg Board. "Egg Product Buyers' Guide." *American Egg Board.* 2013. http://www.aeb.org/pdfs/aeb-buyers-guide.pdf (accessed August 7, 2014).

Ballas Egg Products Corp. *Dried Egg - Specification Sheets.* April 4, 2013. http://www.ballasegg.com/assets/pdfs/2014/specs_dried_2014b.pdf (accessed September 5, 2014).

Brown, Alton. "Angel Food Cake." *Food Network.* 2014. (accessed August 14, 2014).

Chawan, Dhaneshwar B., Carleton G. Merritt, and Wiley W. Hargrove. Microwavable pasta product comprising triethyl citrate and eggs and a process for preparing same. USA Patent US 4990349. 1991.

Chemicalland 21. *Chemistry and Technology of Flavours and Fragrances.* John Wiley & Sons, 2009.

Cho, Ji-Young, Jae-Suk Choi, Se-Eun Kang, Joong-Kyun Kim, Hyun-Woung Shin Shin, and Yong-Ki Hong. "Isolation of antifouling active pyroglutamic acid, triethyl citrate and di-n-octylphthalate from the brown seaweed Ishige okamurae." *Journal of Applied Phycology 17*, 2005: 431-435.

Conrad, Louis Johnson, and Henry Strahley Stiles. Methoed of Improving the Whipping Properties of Gelatin and Gelatin Containing Products and the Resulting Products. USA Patent 2692201. October 19, 1954.

Cotterill, O.J., C.C. Chang, L.E. McBee, and H. Heymann. "Metallic cations affect functional performance of spray-dried heat-treated egg white." *Journal of Food Science* 57 (1992): 1321.

Dsikowitzky, L., J. Schwarzbauer, A. Kronimus, and R. Littke. "The anthropogenic contribution to the organic load of the Lippe River (Germany). Part I: qualitiative characterization of low-molecular weight organic compounds." *Chemosphere* 57 (2004): 1275-1288.

FAO/WHO . *Codex Alimentarius General Standard For Food Additives Online.* 2014. http://www.codexalimentarius.net/gsfaonline/index.html (accessed July 28, 2014).

Fiume, M. "Safety assessment of citric acid, inorganic citrate salts, and alkyl citrate esters as used in cosmetics." *International Journal of Toxicology* 33 (2014): 17-46.

Food and Drug Administration. *Evaluation of the health aspects of citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients.* Washington D.C.: Bureau of Foods, 1977.

—. *Select Committee on GRAS Substances (SCOGS) Opinion: Triethyl citrate.* 1977. http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/ucm261493.htm (accessed July 8, 2014).

Food and Drug Adminstration (FDA). "Requirements for specific standardized eggs and egg products." *21 CFR Part 160 Eggs and Egg Products.* Washington DC, 2014.

Food Safety and Inspection Service. *Egg Products and Food Safety.* Washington DC, 8 6, 2013.

Franks, O.J., M.E. Zabik, and K. Funk. "Angel cakes using frozen, foam-spray-dired, freeze-dried, and spray-dried albumen." *Cereal Chemistry* 46 (1969): 349-356.
Frappier, Edward P., James E. Davis, Martin Grendze, Eric F. Scriven, John T. Wyeth, and Kuen-Wai Chiu. Process for producing citrate esters. US Patent US 6403825 B1. June 11, 2002.
Furia, Thomas E. *CRC Handbook of Food Additives, Second Edition, Volume 1.* CRC Press, 1973.
Garibaldi, J. A., J. W. Donovan, J. G. Davis, and S. L. Cimino. "Heat Denaturation of the Ovomucin-Lysozynie Electrostatic Complex-A Source of Damage to the Whipping Properties of Pasteurized Egg White." *Journal of Food Science 33 (5)*, 1968: 514–524.
International Programme on Chemical Safety. *Triethyl Citrate.* October 18, 1999. http://www.inchem.org/documents/icsc/icsc/eics1350.htm (accessed July 10, 2014).
JECFA. *Joint FAO/WHO Expert Commitee on Food Additives: Triethyl Citrate Monograph.* 1973. http://www.inchem.org/documents/jecfa/jecmono/v14je21.htm (accessed July 21, 2014).
Johnson, Teiko M., and Mary E. Zabik. "Ultrastructural Examination of Egg Albumen Protein Foams." 1981a: 1237–1240.
Johnson, Teiko M., and Mary E. Zabik. "Egg Albumen Proteins Interactions in an Angel Food Cake System." *Journal of Food Science 46*, 1981b: 1231-1236.
Kolah, Aspi K., Navinchandra S. Asthana, Dung T. Vu, Carl T. Lira, and Dennis J. Miller. "Reaction Kinetics of the Catalytic Esterification of Citric Acid with Ethanol." *Industrial & Engineering Chemistry Research 46*, 2007: 3180-3187.
Kolah, Aspi K., Navinchandra S. Asthana, Dung T. Vu, Carl T. Lira, and Dennis J. Miller. "Triethyl Citrate Synthesis by Reactive Distillation." *Industrial & Engineering Chemistry Research 47*, 2008: 1017-1025.
Kothe, Herbert J. Egg white composition. USA Patent US2637654 A. May 5, 1953.
Lee, W.C., and T.C. Chen. "Functional characteristics of egg white solids obtained from papain treated albumen." *Journal of Food Engineering* 51 (2002): 263-266.
Leon, D.M., and E.J. Strandine. Hydrogen peroxide treatment of eggs. US Patent US3364037 A. Jan 16, 1968.
Lomakina, K., and K. Mikova. "A study of the factors affecting the foaming properties of egg white - a review." *Czech Journal of Food Science* 24 (2006): 110-118.
Luck, P.J., and E.A. Foegeding. "The role of copper in protein foams." *Food Biophysics* 3 (2008): 255-260.
Martha Stewart. "Angel Food Cake." *Marth Stewart.* 2014. (accessed August 14, 2014).
McGee, Harold. *On Food and Cooking, First Revised Edition.* New York: Scribner, 2004.
Meyer, R., and N. N. Potter. "Ultrastructural Changes in Unwhipped and Whipped Egg Albumen Containing Sodium Hexametaphosphate and Triethyl Citrate Plus Trisodium Citrate." *Poultry Science 54 (1)*, 1975: 101-108.
Michael Foods, Inc. *Papetti's Egg Whites.* 2013. http://www.michaelfoods.com/foodservice/pdf/EGG482_Egg_Whites_eBrochure_12_13.pdf (accessed September 5, 2014).
Miquelim, Joice N., Suzana C.S. Lannes, and Raffaele Mezzenga. "pH Influence on the stability of foams with protein–polysaccharide complexes." *Food Hydrocolloids 24 (4)*, 2010: 398-405.
Monfort, S., E. Gayan, S. Condon, J. Raso, and I. Alvarez. "Design of a combined process for the inactivation of Salmonella Enteritidis in liquid whole egg at 55 °C." *Journal of Food Microbiology 145 (2-3)*, 2011: 476-482.
Muriana, P.M. "Effect of pH and hydrogen peroxide on heat inactivation of Salmonella and Listeria in egg white." *Food Microbiology* 14 (1997): 11-19.
National Center for Biotechnology Information, U.S. National Library of Medicine. *Compound Summary for: CID 6506.* n.d. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6506#itabs-2d (accessed July 11, 2014).
National Food Corporation. *E.Z. Whip Whites.* 08 04, 2009. http://www.natlfood.com/pdf/E.Z.%20Whip%20Whites.pdf (accessed August 7, 2014).
National Organic Program (NOP). *2013 list of certified USDA organic operations.* Washington DC, 2013.
Oldham, A.M., D.R. McComber, and D.F. Cox. "Effect of cream of tartar level and egg white temperature on angel food cake quality." *Family and Consumer Sciences Research Journal* 29 (2000): 111-124.
OvoPro. *High-Whip Egg Whites Pasteurization Kit.* MOBA. Stationsweg, 2014.
Park, H., M. Misra, L.W. Drzal, and A.K. Mohanty. ""Green" nanocomposites from cellulose acetate bioplastic and clay: effect of eco-friendly triethyl citrate plasticizer." *Biomacromolecules* 5 (2004): 2281-2288.

Penfield, Marjorie P., and Ada Marie Campbell. "IV. Processed Eggs and their Performance in Food Systems." In *Experimental Food Science 3rd Edition*, by Steve Taylor, Marjorie P. Penfield and Ada Marie Campbell. Academic Press, 1990.

Raikos, V., L. Campbell, and S.R. Euston. "Effects of sucrose and sodium chloride on foaming properties of egg white proteins." *Food Research International* 40 (2007): 347-355.

Rowe, David. *Chemistry and Technology of Flavours and Fragrances.* Wiley-Blackwell, 2009.

Royal Society of Chemistry. *ChemSpider Search and Share Chemistry.* 2014. http://www.chemspider.com/Chemical-Structure.13850879.html (accessed July 25, 2014).

Sagis, L.M.C., A.E.A. de Groot-Mostert, A. Prins, and E. van der Linden. "Effect of copper ions on the drainage stability of foams prepared from egg white." *Colloids and Surfaces: Physicochemical and Engineering Aspects*, no. 180 (2001): 163-172.

Shaffer, B.M. Treatment of liquid egg whites. United States Patent US2744829. May 8, 1956.

Sigma-Aldrich. "Triehtyl Citrate Product Specification." *Sigma-Aldrich.* July 9, 2014. (accessed 8 15, 2014).

Silberberg, Martin. *Chemistry. The Molecular Nature of Matter and Change.* St. Louis: Mosby-Year Book, Inc., 1996.

Soccol, Carlos R., Luciana P.S. Vandenberghe, Cristine Rodrigues, and Ashok Pandey. "New Perspectives for Citric Acid Production and Application." *Food Technology Biotechnology 44(2)*, 2006: 141-149.

Stackelberg, P.E., J. Gibs, E.T. Furlong, M.T. Meyer, S.D. Zaugg, and R.E. Lippincott. "Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds." *Science of the Total Environment* 377 (2007): 255-272.

Triller, Benjamin, and Matthias Hüttl. Use of triethyl citrate as a denaturing agent for ethanol. Europe Patent EP2735301 A1. May 28, 2014.

United States Pharmacopeial Convention. *Food Chemicals Codex.* Washington D.C., 2012.

Vertellus. "Triethyl Citrate Safety Data Sheet." *Vertellus.* June 5, 2014. (accessed 8 15, 2014).

World Health Organization. *Triethyl citrate.* Evaluation of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA), 1991: World Health Organization, n.d.

Ziegler, Homer F., and Henry J Buehler. USA Patent US3219457 A. 1965.