# Yeast

## Handling/Processing

### Identification of Petitioned Substance

This technical report discusses yeasts for use in organic handling and processing. The scope of this yeast report includes but is not limited to those listed on Table 1. Single cell organisms, such as yeast, do not commonly have a CAS number or chemical name and are instead referenced by their species and/or trade name. Yeast uses in production and handling include their use in fermentation, baking, nutrition, probiotics, and production of yeast extracts. The types of yeast discussed in this technical report include, but are not limited to, autolysate, bakers, brewers, nutritional, and smoked.

**Table 1.** Yeast used in organic production and handling (Bekatorou, Psarianos and Koutinas 2006; Czeruka, Piche and Rampal 2007)

| *Previous name | |
|---|---|
| **Common Name** | **Trade Names** |
| *Saccharomyces cerevisiae* (CAS# 68876-77-7) | Red Star®, Active Dry Yeast, Baker's Cream Yeast (Baker's yeast) /Faex, Medicinal Yeast, Levure (Brewers yeast supplement)/Augustiner, W 177, BSI-72 (Brewer's yeast brewing) /VIN 7, WE 372, ICV D-47 (Wine yeast) |
| *Candida utilis (*Torulopsis utilis)* | Lake States®, Torula Yeast |
| *Saccharomyces uvarum (*Saccharomyces carlsbergensis)* | NRLL Y-1347 (Brewing Yeast /Wine Yeast) |
| *Saccharomyces bayanus* | Premier Cuvee, PDM, EC 1118, Prise de Mousse (Wine Yeast) |
| *Saccharomyces chevalieri* | Wine Yeast |
| *Saccharomyces fructuum* | Wine Yeast |
| *Saccharomyces pasteurianus* | Wine Yeast |
| *Saccharomyces sake* | Wine Yeast |
| *Saccharomyces vini* | Wine Yeast |
| *Saccharomyces boulardii* | Florastor®, OptiBac (Probiotic yeast) |
| *Kluyveromyces lactis* | Whey Yeast |
| *Kluyveromyces marxianus (*Kluyveromyces fragilis)* | Whey Yeast |
| *Schizosaccharomyces pombe* | Probiotic yeast |

### Summary of Petitioned Use

Yeast is listed on the National List at §205.605(a) as an allowed nonsynthetic with the annotation "when used as food or a fermentation agent in products labeled as 'organic,' yeast must be organic if its end use is for human consumption; nonorganic yeast may be used when organic yeast is not commercially available. Growth on petrochemical substrate and sulfite waste liquor is prohibited. For smoked yeast, nonsynthetic smoke flavoring process must be documented." Section §205.605 identifies nonagricultural substances allowed for use as ingredients in or on products to be labeled as "organic" or "made with organic (specified ingredients or food group(s))." (Electronic Code of Federal Regulations: Part 205-National Organic Program 2013). Yeast may not be used in organic production and handling if produced via genetic modification/engineering (§205.105(c) & (e)).

## Characterization of Petitioned Substance

**Composition of the Substance:**

Yeast are single celled microorganisms that can produce enzymes, carbon dioxide ($CO_2$), and other metabolites from carbohydrates, whose functional roles are frequently used in the processes of fermentation, baking, flavoring foods, adding nutritional value, and providing health benefits (Evans, Heritage and Killington 2000; Dubey, Maheshwari and Saravanamurthu 2010).

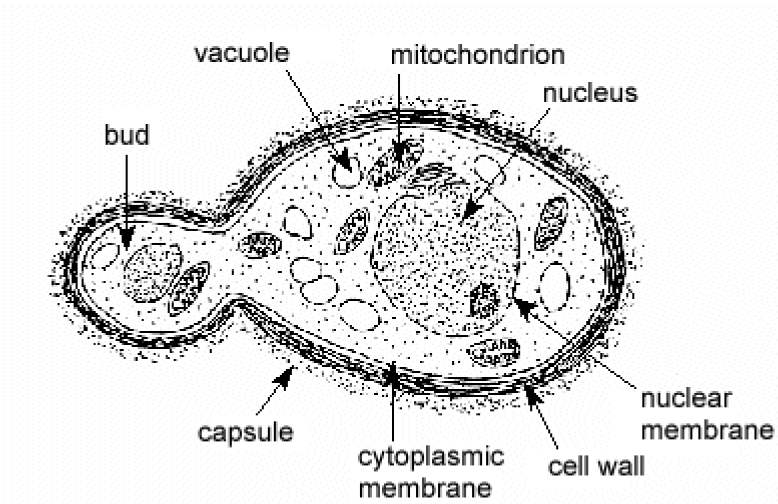


Figure 1. Yeast cell components and structure (Northern Arizona University 2008).

The interiors of yeast cells are comprised of amino acids, peptides, carbohydrates, salts (Hassan 2011), and monosodium glutamate (MSG) (Dubey, Maheshwari and Saravanamurthu 2010). The cell wall is composed of glucan, a glycoprotein, mannoprotein (Alexandre and Guilloux-Benatier 2006), and chitin (Kollar, et al. 1997). Additionally, yeast contains many types of enzymes, cofactors, and "nucleic acid, particularly RNA, which is obtained from *Candida utilis* and *Saccharomyces sp.*" (Dubey, Maheshwari and Saravanamurthu 2010).

Literature commonly does not identify specific strains of yeast species, such as *Saccharomyces cerevisiae*. For example, though many different strains of *Saccharomyces cerevisiae* exist, and some are only present in specific regions, all strains are commonly referred to in literature as *Saccharomyces cerevisiae* (Cornuet, Karst and Legras 2007). Specific strains appear to be clearly identified in some literature discussing taxonomy, such as *Saccharomyces cerevisiae Y-12632*$^{NT}$ and *Schizosaccharomyces pombe* Y-12796$^{T}$ (Kurtzman, Fell and Boekhout 2011), and when articles discuss genetically modified strains of yeast, such as *Saccharomyces cerevisiae* strains ML01 and P1Y0 (U.S. FDA 2013).

**Source or Origin of the Substance:**

Yeast, such as *Saccharomyces uvarum* and *Saccharomyces cerevisiae,* are ubiquitous in the environment, with many sources depicted in Table 2. They have also been isolated from sources with a high concentration of carbohydrates (sugars), including phyllosphere, grains, fruits, honey, soil, and plant surfaces (U.S. Environmental Protection Agency 1997). Typically, specific yeast strains are grown in a lab environment and used to inoculate growth media for industrial production, as opposed to being gathered from the environment to prevent contamination from undesirable or pathogenic microorganisms (Dubey, Maheshwari and Saravanamurthu 2010).

**Table 2.** Origin of yeast strains and their uses (Cornuet, Karst and Legras 2007) Strain origin and grouping. The number of strains in the population used for $F_{ST}$ tree[1] is lower as the first group may include several times the same genotype, or because only strains from a well-defined geographical origin are retained for further analysis (i.e., Tarrogona strains among Spanish strains, or Firenze strains among Italians)

| Origin | Number of analyzed strains | Population' for $F_{ST}$ tree | No. of strains in the population |
|---|---|---|---|
| Alpechin, Spain | 1 | | |
| Ale beer miscellaneous (France, Belgium, England, The Netherlands …) | 8 | Ale Beer | 8 |
| Bread, Italy Sicily | 20 | Bread, Italy, Sicily | 19 |
| Bread, miscellaneous (France, Japan, Spain …) | 9 | Bread miscellaneous | 9 |
| Cassava and banana, Burundi | 2 | | |
| Cheese, France Camembert | 2 | Fermented milk | 14 |
| Cheese, France Cantal | 12 | Fermented milk | 14 |
| Cider, France Brittany | 8 | | |
| Distillery, Australia | 1 | | |
| Distillery, Brazil | 8 | Distillery, Brazil | 8 |
| Distillery, China | 8 | Distillery, China | 7 |
| Fermented milk, Morocco | 1 | Fermented milk | 14 |
| Fruit, Indonesia | 1 | | |
| Grapes (*Vitis amurensis*), Russia | 1 | | |
| Laboratory strains (USA, France …) | 8 | | |
| Lagar beer miscellaneous (France, China, USA …) | 15 | | |
| Miscellaneous, Japan | 1 | | |
| Natural resources, Vietnam | 5 | | |
| Oak exudates, USA | 2 | | |
| Palm wine, Ivory Coast | 1 | | |
| Palm wine, Nigeria | 20 | Palm wine (Nigeria) | 19 |
| Rice wine, China miscellaneous | 6 | Rice wine | 10 |
| Rice wine, Laos | 3 | Rice wine | 10 |
| Rice wine, Thailand | 1 | Rice wine | 10 |
| Rum, France French Indies | 15 | Rum French Indies | 15 |
| Sake, Japan | 14 | Sake (Japan) | 11 |
| Sorghum beer, Ghana | 4 | | |
| Trout guts, Norway | 1 | | |
| Type strain CBS1907 (Italy) | 1 | | |
| Wine and fruits, Turkey | 7 | | |
| Wine, Australia | 4 | | |
| Wine, Austria | 17 | Wine Austria | 13 |

[1] $F_{ST}$ is a statistical test used for determining whether two groups of data are statistically different. In this example, it is used to determine the genetic distance between yeast strains. Genetic distance identifies how similar strains are to one another. A large genetic difference indicates a large divergence in genes from a common ancestor.

| | | | |
|---|---|---|---|
| Wine, Croatia | 5 | | |
| Wine, France Alsace | 100 | Wine France Alsace | 71 |
| | | Wine France Alsace 'Central Europe Group | 14 |

**Properties of the Substance:**

Yeasts are single celled eukaryotic microorganisms in the kingdom Fungi (Bennett 1998; Ingraham 2010). Eukaryotes are cells which have a nuclear membrane and cell walls. Fungi lack chloroplasts unlike plants, and are heterotrophs, a type of organism that consumes living and dead organic materials for energy and food (Bennett 1998). Fungi accomplish this task by releasing proteolytic, glycolytic, or lipolytic enzymes to digest organic matter, or by absorbing small molecules such as amino acids and simple sugars through their cell wall (Baron 1996). The Kingdom Fungi includes both macroscopic organisms such as mushrooms, and microscopic organisms such as yeast and mold (Ingraham 2010). Yeasts, unlike molds and mushroom fungi, exist as individual cells instead of forming "filamentous, vegetative cells called hyphae," which are able to be interconnected with other cells as a multi-cellular body (Baron 1996).

Yeasts have two primary methods of reproducing: budding, and fission. Budding occurs when the parent cell enlarges and a protrusion forms along the cell wall, forming a bud. The bud eventually either breaks off from the parent cell, or remains partially conjoined via elongated cells forming a pseudo mycelium, a small multicellular structure. The dimorphic type of fungi and yeasts which produce septate are "capable of adapting their structures to changes in their environment" and forming mycelia instead of pseudomycelia (Evans, Heritage and Killington 2000). Fission is another form of yeast cell division, where a parent cell divides into two daughter cells in a manner similar to the "transverse binary fission seen in bacterial reproduction" (Evans, Heritage and Killington 2000).

Yeast species are generally grown under aerobic (presence of oxygen) conditions, and fermented under anaerobic (lack of oxygen) conditions, which in the presence of sugar produces ethanol (Bekatorou, Psarianos and Koutinas 2006).

"Morphologically, *Saccharomyces uvarum* has been described as spheroidal, ovoid, ellipsoidal, cylindrical or elongate in shape. There are also some filamentous forms. The nonfilamentous forms can occur singly, in pairs, or clusters. Different strains of this species of yeast have been determined by the size of the individual cells" (U.S. Environmental Protection Agency 1997). "Yeasts are able to grow in foods with a low pH (5.0 or lower) and in the presence of sugars, organic acids and other easily metabolized carbon sources" (Kurtzman, 2006), and they have a very high tolerance to the presence of "high sucrose, ethanol, acetic acid, sorbic acid, benzoic acid and sulfur dioxide" (Dubey, Maheshwari and Saravanamurthu 2010). Yeast produce many types of enzymes, including invertase, β-glycosidase, alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and hexokinase (Dubey, Maheshwari and Saravanamurthu 2010).

**Specific Uses of the Substance:**

*Baker's Yeast*

Strains of yeast that are used as leavening agents from the production of $CO_2$ are referred to as baker's yeast (Dubey, Maheshwari and Saravanamurthu 2010). Different varieties of baker's yeast are available for use in both commercial bakeries and for home use. *Saccharomyces exiguus* "is a wild yeast found on plants, fruits and grains that is occasionally used for baking" (Dubey, Maheshwari and Saravanamurthu 2010). Active dry yeast, which contains a single strain of *Saccharomyces cerevisiae*, is used in the production of leavened bread (Bekatorou, Psarianos and Koutinas 2006). Inactive dry yeast is used solely for adding flavor or the "conditioning of dough properties" without leavening bread or requiring rehydration for activation (Bekatorou, Psarianos and Koutinas 2006). The particular strains of *Saccharomyces cerevisiae* that are used in baking produce large quantities of $CO_2$, "tolerate high-levels of sucrose, endure freeze-thawing stress, and rapidly utilize maltose." Additionally, baker's yeast lacks the enzyme α-galactosidase, unlike brewer's yeast, which allows melibiase to be broken down[2]. *Torulaspora delbrueckii* is used in frozen dough for its high tolerance to the freezing/thawing process (Kurtzman, Fell and Boekhout 2011).

*Brewer's Yeast*

Strains of yeast which include the enzyme α-galactosidase are used in the anaerobic fermentation process, which converts sugar into ethanol, and are commonly referred to as brewer's yeast (Kurtzman, Fell and Boekhout 2011). Alcoholic beverages for which brewer's yeast is responsible are split into two categories: fermented (beer and wine), and distilled (spirits and liqueurs) (Soccol, Pandey and Larroche 2013). .

*Saccharomyces uvarum* and *Saccharomyces cerevisiae* are both used in the fermentation process for a variety of alcoholic beverages (Bekatorou, Psarianos and Koutinas 2006). *Saccharomyces cerevisiae*, for example, is typically used "in the production of ales, porters, stouts, wheat beers, etc." while *Saccharomyces uvarum* is commonly used in the production of "lager, beers like Pilsners, Bocks, American malt liquors, etc. (Bekatorou, Psarianos and Koutinas 2006)."

*Nutritional Yeast*

*Saccharomyces cerevisiae* is the most common species of yeast that, when deactivated, is consumed for its nutritional content. It is generally referred to by the common name "nutritional yeast" (Dubey, Maheshwari and Saravanamurthu 2010). Nutritional yeast has a high concentration of vitamins, protein, and B vitamins, provides flavor, and is low in salt and fat (Dubey, Maheshwari and Saravanamurthu 2010). "Nutritional yeast has nutty, cheesy and creamy flavors which makes it popular as an ingredient in cheese substitutes. It is often used by vegans in place of parmesan...and as a topping for popcorn" (Dubey, Maheshwari and Saravanamurthu 2010).

*Probiotic Yeast*

Probiotics are characterized as microorganisms which exhibit beneficial effects in the "prevention and treatment of specific pathological conditions" when ingested (Czerucka, Piche and Rampal 2007). *Saccharomyces bouldarii* has been shown to be effective in double blind studies as a probiotic (Czerucka, Piche and Rampal 2007). It has been tested and shown to be effective in treating patients with diarrhea and irritable bowel diseases, such as moderately advanced Crohn's disease. Additionally, this strain of yeast exhibits a resistance to antibiotics, and can be used effectively by individuals who are consuming antibiotic medication. *Saccharomyces bouldarii* also partially or completely neutralizes toxins derived from certain strains of bacteria, such as *V. cholera* and *C. difficile* (Czerucka, Piche and Rampal 2007).

*Smoked Yeast*

Yeast which has been smoked primarily to increase its palatability is commonly called smoked yeast (Freshel 1954). It is used both as a substitute for other foods and for direct consumption (Freshel 1954). Methods for producing smoked yeast may impart flavors such as mint, chocolate, clove, cinnamon, basil, and traditional smoke flavoring (Freshel 1954). Though the methods to produce these flavors exist, not all flavors may currently be available to the consumer; traditional smoke flavoring is the most prevalent (Freshel 1954).

*Yeast Autolysate*

Yeast autolysate, also known under the name yeast extract, is comprised of the "water-soluble components of the yeast cell, the composition of which is primarily amino acids, peptides, carbohydrates, salts" (Hassan 2011) and monosodium glutamate (MSG) (Dubey, Maheshwari and Saravanamurthu 2010). Yeast autolysates are concentrates of the soluble components of yeast cells and are generally produced by autolysis, a process by which the cell wall is induced mechanically or chemically to rupture. Nitrogen (N) components and vitamins are the value of yeast extract because of their nutritional characteristics (Hassan 2011). Hence, yeast extract has been mainly used in the food industry, as a flavoring agent in soup, sauces, gravies, stews, snack food and canned food, as well as in pet foods and cosmetic materials and as a plant

[2] α-Galactosidase is also known as melibiase. Melibiase is responsible for hydrolyzing melibiose, a disaccharide, into galactose and glucose (European Molecular Biology Laboratory: The European Bioinformatics Institute 2013).

nutrient. Other applications include vitamin and protein supplements in health foods and as a source of nutrients in microbiological media (Hassan 2011). Additionally, yeast autolysate exhibits antioxidant properties and stimulates the immune system, implying that it may also be used for medicinal purposes (Hassan 2011). When used as a flavoring agent in organic production, yeast autolysate is considered a natural flavor according to the FDA definition. Therefore, yeast autolysate could potentially be assessed using either the annotation for yeast or for nonsynthetic flavors under USDA organic regulations section 205.605(a).

*Torula Yeast*

*Candida utilis,* a species of yeast commonly referred to as *Torula*, or *Candida* yeast, is consumed for its high nutrient content, and as a meat substitute or food additive (Bekatorou, Psarianos and Koutinas 2006). *Torula* yeast is produced via fermentation, "harvested, washed, thermalized[3] and spray dried" (Bekatorou, Psarianos and Koutinas 2006). *Torula* yeast is described as "a highly digestible and nutritious food, containing more than 50% of protein (rich in lysine, threonine, valine and glutamic acid), minerals and vitamins (mainly niacin, pantothenic acid and B vitamins). *Torula* yeast can also be used as a meat substitute due to its slightly yeasty and meaty flavors, or as a food additive in many processed foods, in seasonings, spices, sauces, soups, dips, *etc.* It is also used in vegetarian and diet food, in baby food, meat products, dough, etc. (Bekatorou, Psarianos and Koutinas 2006).

*Chromium Yeast*

When yeast is grown in a medium fortified by chromium salts (e.g. $CrCl_3 \bullet 6H_2O$), chromium is adsorbed into the yeast and ingested as a trace mineral supplement (Skogerson 1982) (Stearns 2000). Saccharomyces uvarum when not fortified naturally contains low concentrations of chelated chromium at 2 to 4 µg/g yeast solids (Skogerson 1982). Studies are not conclusive, but there is some indication that chromium may not be an essential trace mineral, and instead acts as a drug (Stearns 2000). The study depicted a correlation between chromium and iron metabolism, where increases in $Cr^{3+}$ led to a reduction of iron (Stearns 2000).

*Selenium Yeast*

High-selenium yeast is grown in a growth medium fortified by inorganic selenium salts (Kamel 2013). It is allowed as an ingredient in "processed fruit and fruit juices, processed vegetables and vegetable juices, commercial soups and soup mixes" (FDA 2013), yogurts, breads, instant cereals, breakfast and granola type bars, beverages, pastas, crackers, salty snacks, pretzels and popcorn at levels of 5 to 60 mg/kg, and in medical foods such that the daily intake will not exceed 19.2 mg/day (FDA 2013).

*Miscellaneous or Other Yeast Products*

Yeast glycan, which is derived from the cell wall after autolysis, is obtained via alkali extraction, a synthetic process (Aleck, et al. 1975). Yeast glycan can be used as a low calorie fat substitute for specific types of food "such as salad dressing, ice cream, puddings, sour cream based dips, etc." (Aleck, et al. 1975). Yeast mannoprotein, also derived from the cell wall, can be used in winemaking as an alternative to commercial mannoproteins to "reduce red wine astringency and increase the smoothness and body" of wine, while retaining the color (Guadalupe, Martinez and Ayestaran 2010).

Kombucha is a black or green tea containing sugar that has been fermented with "tea fungus," a combination of yeast and acetic acid bacteria (Battikh, Bakhrouf and Ammar 2012), that is consumed for both its flavor and health benefits. "Yeast has synergistic effects when used with acetic acid bacteria in the preparation of kombucha, a fermented sweetened tea " (Dubey, Maheshwari and Saravanamurthu 2010). Species of yeast used in Kombucha include *"Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata, Saccharomyces cerevisiae, Zygosaccharomyces baili, Torulaspora delbrueckii, Brettanomyces bruxellensis, Brettanomyces lambicus, Brettanomyces custersii, Candida stellata,* and other species of *Candida* and *Pichia* have been identified" (Battikh, Bakhrouf and Ammar 2012). Kombucha has been shown to exhibit antimicrobial, antifungal activity (Battikh, Bakhrouf and Ammar 2012), which includes the inhibition of pathogens such as *"Staphylococcus aureus*, *Shigella sonnei, Escherichia coli, Aeromonas hydrophilia, Yersinia enterolitica,*

[3] Thermolysis is a process by which yeast is heated to render it inactive and incapable of fermentation (Bekatorou, Psarianos and Koutinas 2006).

*Pseudomonas aeruginosa, Enterobacter cloacae, Staphylococcus epidermis, Campylobacter jejuni, Salmonella enteritidis, Salmonella typhimurium, B. cereus, Helicobacter pylori,* and *Listeria monocytogenes"* (Mo, Zhu and Chen 2008).

**Table 3**. Species of yeast used in various types of food (Kurtzman, Fell and Boekhout 2011).

| Food | Yeast Species Used | Food | Yeast Species Used |
|---|---|---|---|
| **Beers and ales** | *Saccharomyces cerevisiae* | **Fermented olives and cucumbers** | *Debaryomyces spp.* |
| | *Saccharomyces bayanus* | | *Candida spp.* |
| | *Saccharomyces pastorianus* | | *Kluyveromyces marxianus* |
| | *Saccharomyces bayanus var. uvarum* | | *Lachancea cidri* |
| **Breads and bakery products** | *Saccharomyces cerevisiae* | | *Pichia spp.* |
| | *Torulaspora delbrueckii* | | *Rhodotorula spp.* |
| | *Candida krusei (sourdough)* | | *Saccharomyces spp.* |
| | *Candida kmilleri (sourdough)* | **Kenkey (African fermented maize)** | *Candida spp.* |
| | *Kazachstania exigua (sourdough)* | | *Debaryomyces spp.* |
| | *Zygosaccharomyces rouxii* | | *Kluyveromyces spp.* |
| **Cachaca** | *Saccharomyces cerevisiae* | | *Saccharomyces spp.* |
| | *Schizosaccharomyces pombe* | | *Trichosporon spp.* |
| **Cheeses** | *Debaryomyces hansenii* | **Kimchi** | *Candida spp.* |
| | *Candida spp.* | | *Cryptococcus spp.* |
| | *Kluyveromyces marxianus* | | *Debaryomyces spp.* |
| | *Kluyveromyces lactis* | | *Saccharomycopsis spp.* |
| | *Yamowia Jipolytica* | | *Kluyveromyces spp.* |
| | *Geotrichum candidum* | | *Pichia spp.* |
| | Other *Geotrichum spp.* | | *Rhodotorula spp.* |
| | *Rhodotorula spp.* | | *Saccharomyces spp.* |
| | *Trichosporon spp.* | **Lao Chao** | *Saccharomycopsis fibuligera* |
| | *Saccharomyces cerevisiae* | | *Saccharomycopsis malanga* |
| **Other dairy products (e.g., kefir, yogurt, fermented milk)** | *Kluyveromyces lactis* | **Soy paste (Chiang; Miso)** | *Zygosaccharomyces rouxii* |
| | *Kluyveromyces marxianus* | | *Candida spp.* |
| | *Candida kefyr* | **Soy sauce (Jiang yu; Shoyu)** | *Zygosaccharomyces rouxii* |
| | *Candida famata* | | *Candida famata* |
| | *Candida krusei* | | *Candida etchellsii* |
| | *Debaryomyces hansenii* | | *Candida versatilis* |
| | *Geotrichum candidum* | | *Debaryomyces spp.* |
| | *Yarrowia lipolytica* | | Other *Candida spp.* |
| | *Saccharomyces cerevisiae* | **Tea fungus** | *Zygosaccharomyces* |

| | | | |
|---|---|---|---|
| | | | *Kombuchaensis* |
| **Ciders** | *Saccharomyces cerevisiae* | | *Candida spp.* |
| | *Saccharomyces bayanus* | **Wines** | *Saccharomyces cerevisiae* |
| **Cocoa** | *Saccharomyces cerevisiae* | | *Saccharomyces bayanus* |
| | *Hanseniaspora uvarum* | | *Saccharomyces bayanus var. uvarum* |
| | *Kloeckera apiculata* | | *Saccharomyces kudriavezii* |
| | *Kluyveromyces marxianus* | | *"Saccharomyces sake"* |
| | *Pichia fermentans* | | Other *Saccharomyces spp.* |
| **Coffee** | *Kluyveromyces marxianus* | | *Hanseniaspora uvarum* |
| | *Saccharomyces bayanus* | | *Kloeckera apiculata* |
| | *Schizosaccharomyces spp.* | **Silage** | *Candida spp.* |
| | *Candida boidinii* | | *Cryptococcus spp.* |
| **Fermented meats and sausages** | *Debaryomyces hansenii* | | *Pichia spp.* |
| | *Candida spp.* | | *Saccharomyces spp.* |
| | *Cryptococcus spp.* | | *Trichosporon spp.* |
| | *Rhodotorula spp.* | **Probiotics** | *Saccharomyces boulardii* |
| | *Saccharomyces cerevisiae* | | *Saccharomyces cerevisiae* |
| | *Yarrowia lipolytica* | | *Kluyveromyces marxianus* |
| **Fermented milks** | *Kluyveromyces spp.* | | *Candida spp.* |
| | *Candida parapsilosis* | | |
| | *Candida krusei* | | |
| | *Candida valida* | | |
| | *Saccharomyces bayanus* | | |
| | *Yarrowia lipolytica* | | |

**<u>Approved Legal Uses of the Substance:</u>**

Yeast appears in FDA regulations as food additives permitted for direct addition to food for human consumption at 21CFR Chapter 1, subchapter B, part 172.896 (FDA 2013). Subchapter A of the same section outlines general provisions for direct addition to food. Dried yeast (*Saccharomyces cerevisiae* and *Sacharomyces fragilis* ) and dried torula yeast (*Candida utilis* ) both appear on the FDA's list of food additives permitted for direct addition to food for human consumption, with the annotation "may be safely used in food provided the total folic acid content of the yeast does not exceed 0.04 milligram per gram of yeast" (approximately 0.008 milligram of pteroyglutamic acid per gram of yeast) (FDA 2013). A total of seven yeast products or byproducts carry GRAS notifications (FDA 2013). Three of these notices pertain to genetically modified yeasts, two pertain to yeast byproducts including baker's yeast mannoprotein and baker's yeast beta-glucan, and two relate to selenium yeast products (FDA 2013).

USDA

Yeast is included at 205.605(a) in the National Organic Program Rule allowed as ingredients in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))."

EPA

The EPA has identified yeast extract hydrolysate from *Saccharomyces cerevisiae* as exempt from the requirement of tolerance on all food commodities when used for suppression of plant diseases (EPA 2004).

**<u>Action of the Substance:</u>**

The action of yeast varies depending on the nature of the various end products being produced, such as breads, alcoholic beverages, flavor enhancers and nutritional supplements.

*Baker's yeast*

In bread making, yeasts, notably strains of *Saccharomyces cerevisiae*, produce carbon dioxide gas via aerobic fermentation that expands the dough during various production stages, including "ripening" and in the beginning stages of baking (Cauvain 2003). In this way, the yeast acts as a leavening agent (Rose and Vijayalakshmi 1993). Yeast play three major roles in bread making: to increase the volume of dough by producing carbon dioxide via alcoholic fermentation of sugars present in the dough, to bring about a change in structure and texture in the dough as a result of the carbon dioxide bubbles, and to add flavor to the bread (Rose and Vijayalakshmi 1993).

*Brewer's yeast*

The term brewer's yeast refers both to the yeast used in the fermentation of alcoholic beverages and to the nutrient rich, post-fermentation spent yeast. For the purpose of this report, the action of brewer's yeast as described below is applicable to all types of alcoholic beverages (i.e., cider, beer, wine and spirits).

In brewing the principle action of the yeast is the production of alcohol (Boulton and Quain 2001). While cultured yeast is most often used presently, some fermentation techniques still rely on traditional methods of using wild yeasts that are naturally present on fruits or processing equipment to accomplish alcoholic fermentation (Watson 1993; Beech 1993). In the production of alcohol, yeast metabolize sugary wort (liquid extracted from grain) and convert the wort into alcohol and carbon dioxide (Hammond 1993). The unviable, spent yeast's principle action is nutritive when separated from the spent growth media (e.g., beer) (Lodolo, et al. 2008) and, when used to manufacture yeast extracts, it is considered a raw material (Vieira, et al. 2012). Normally the inactive yeast sold as brewer's yeast is not, however, manufactured as a byproduct of the brewing industry, but is produced via fermentation on enriched cane or beet molasses substrate (Bekatorou, Psarianos and Koutinas 2006). Brewer's yeast is high in vitamins and minerals such as Vitamin B, zinc, chromium, iron, magnesium, folic acid, and biotin and is often sold as a supplement in tablet, powders, flakes or in liquid form (Bekatorou, Psarianos and Koutinas 2006). Chromium levels are also high in brewer's yeast and may contribute to lowering of glucose levels in the body. (Mooradian, et al. 1994).

*Nutritional yeast*

Nutritional yeast contains free glutamic acid, which enhances the ability of taste buds to taste savory foods. It also contains vitamin B, zinc, chromium, iron, magnesium, folic acid, and biotin (Red Star Yeast 2013).

*Smoked yeast*

Smoked yeast is used as a flavor enhancer to impart a smoke flavor similar to smoked meats (Freshel 1954)

*Yeast Autolysate*

Yeast autolysate is used as a flavor enhancer due to elevated levels of glutamic acid, a nonessential amino acid that helps taste buds to detect savory foods. Yeast extract used for flavor enhancement can replace glutamates and nucleotides in many processed foods (Bekatorou, Psarianos and Koutinas 2006). Yeast autolysate used primarily as a flavor enhancer in organic foods would potentially be assessed according to the listing "flavors –nonsynthetic" and not according to the "yeast" listing at §205.605(a). It is also used as growth media.

*Probiotic yeast:*
Several yeast species are identified as having positive probiotic effects on the digestive tracks of humans. (Hatoum, Labrie and Fliss 2012). However, *Saccharomyces cerevisiae* var. *boulardii* is the only yeast with documented probiotic effects (Sazawal, et al. 2006). These effects include the prevention of bacterial adherence and translocation in intestinal epithelial cells, production of factors that neutralize bacterial toxins, and modulation of the host cell signaling pathway associated with pro-inflammatory response during bacterial infection (Moslehi-Jenabian, Lindegaard and Jespersen 2010).

*Chromium yeast*
Chromium is identified as a component of glucose tolerance factor (GTF), a dietary ingredient required for optimal glucose utilization (Hambridge K 1974).

*Selenium yeast*
Yeast grown on selenium rich substrate incorporates a large amount of selenium into its biomass (Rayman 2000). High-selenium yeast is allowed as an ingredient in "processed fruit and fruit juices, processed vegetables and vegetable juices, commercial soups and soup mixes" (FDA 2013), yogurts, breads, instant cereals, breakfast and granola type bars, beverages, pastas, crackers, salty snacks, pretzels and popcorn at levels of 5 to 60 mg/kg, and in medical foods such that the daily intake will not exceed 19.2 mg/day (FDA 2013).

**Combinations of the Substance:**

While some *Saccharomyces cerevisiae* species used for beer making are not formulated with other ancillary substances (Lallemand 2010), many commercially available yeasts are formulated with other ingredients. Ancillary ingredients are those ingredients (e.g. carriers, stabilizers and antioxidants) that are combined with the "active" ingredient or substance listed on the National List to provide a ***necessary*** technical effect on the National List substance. An example of such an ancillary ingredient is ascorbic acid, a National List substance listed at §205.605(b) which is added to yeast as a preservative (Lallemand Inc. 1998).

Ancillary ingredients not appearing on the National List are routinely combined with yeast on a commercial scale. Other ingredients including water, emulsifiers and cutting oils (often soybean or cotton oil) are added to the final yeast product to aid in shaping (Bekatorou, Psarianos and Koutinas 2006; Lallemand Inc. 1996). These emulsifiers are added to the final yeast cake product to impart a white, creamy appearance and to inhibit water spotting on yeast cakes (Office of Air Quality Planning and Standards 1995; Lallemand Inc. 1996). The small amount of oil used is added to extrude, or to force the yeast out of nozzles to form ribbons of yeast cake (Office of Air Quality Planning and Standards 1995).

While the specific emulsifiers used are often unidentified in literature, sorbitan monostearate (E Number E491), sorbitan tristearate (E Number E 492) sorbitan monolaurate (E Number 493), sorbitan monooleate (E number 494), and sorbitan monopalmitate (E Number 495) are identified as emulsifiers used to formulate dry yeast and yeast for baking (Fermentis 2012; European Food Emulsifier Manufacturers' Association 2013).

**Table 4.** Emulsifiers used in yeast for baking (European Food Emulsifier Manufacturers' Association 2013)

| Substances | Manufacturing process |
|---|---|
| Sorbitan monostearate (E491) and sorbitan tristearate (E492) | Manufactured through the esterification of sorbitol with commercial stearic acid derived from food fats and oils |
| Sorbitan monolaurate (E493) | Manufactured though the esterification of sorbitol with lauric acid derived from food fats and oils |

| | |
|---|---|
| Sorbitan monooleate (E494) | Manufactured through the esterification of sorbitol with commercial oleic acid derived from food fats and oils |
| Sorbitan monopalmitate (E494) | Manufactured by the esterification of sorbitol with commercial palmitic acid derived from food fats and oils |

In addition to emulsifiers, ascorbic acid (mentioned above) and antioxidants are also added depending on the type of yeast product produced (Lallemand Inc. 1998). Such antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) (Ackerman and Pomper 1969; FDA 2013). Furthermore, various materials are identified by the FDA as permitted additives in yeast manufacturing and are included in Table 5.

**Table 5**. Materials identified by FDA as secondary direct food additives permitted in food for human consumption under Subpart D-specific usage additives at 21 CFR §173.340

| **Substances** | **Limitations** | **Function** |
|---|---|---|
| Aluminum stearate | As defined in §172.863 of this chapter. | Component of defoaming agent |
| Butyl stearate | | Component of defoaming agent |
| BHA | As an antioxidant, not to exceed 0.1 percent by weight of defoamer. | Component of defoaming agent |
| BHT | Do. | Component of defoaming agent |
| Calcium stearate | As defined in §172.863 of this chapter. | Component of defoaming agent |
| Fatty acids | As defined in 172.860 of this chapter. | Component of defoaming agent |
| Formaldehyde | As a preservative. | Component of defoaming agent (preservative) |
| Hydroxylated lecithin | As defined in 172.814 of this chapter. | Component of defoaming agent |
| Isopropyl alcohol | | Component of defoaming agent |
| Magnesium stearate | As defined in 172.863 of this chapter. | Component of defoaming agent |
| Mineral oil: Conforming with 172.878 of this chapter | Not more than 150 ppm in yeast, measured as hydrocarbons. | Component of defoaming agent |
| Odorless light petroleum hydrocarbons: Conforming with 172.884 of this chapter | | Component of defoaming agent |
| Petrolatum: Conforming with 172.880 of this chapter | | Component of defoaming agent |
| Petroleum wax: Conforming with 172.886 of this chapter | | Component of defoaming agent |
| Petroleum wax, synthetic | | Component of defoaming agent |
| Polyethylene glycol (400)dioleate: Conforming with 172.820(a)(2) of this chapter and providing the oleic acid used in the production of this substance complies with 172.860 or 172.862 of this chapter | As an emulsifier not to exceed 10 percent by weight of defoamer formulation. | Component of defoaming agent |

| | | |
|---|---|---|
| Synthetic isoparaffinic petroleum hydrocarbons: Conforming with 172.882 of this chapter | | Component of defoaming agent |
| Oleic acid derived from tall oil fatty acids | Complying with 172.862 of this chapter. | Component of defoaming agent |
| Oxystearin | As defined in 172.818 of this chapter. | Component of defoaming agent |
| Polyoxyethylene (600) dioleate | | Component of defoaming agent |
| Polyoxyethylene (600) monoricinoleate | | Component of defoaming agent |
| Polypropylene glycol | Molecular weight range, 1,200-3,000. | Component of defoaming agent |
| Polysorbate 80 | As defined in 172.840 of this chapter. | Component of defoaming agent |
| Potassium stearate | As defined in 172.863 of this chapter. | Component of defoaming agent |
| Propylene glycol mono- and diesters of fats and fatty acids | As defined in 172.856 of this chapter. | Component of defoaming agent |
| Soybean oil fatty acids, hydroxylated | | Component of defoaming agent |
| Tallow, hydrogenated, oxidized or sulfated | | Component of defoaming agent |
| Tallow alcohol, hydrogenated | | Component of defoaming agent |

## Status

**Historic Use:**

Yeast has been used in the production of wine, beer, and bread for thousands of years, with the oldest known remains of wine dated at over 7,000 years old (Alba-Lois and Segal-Kischinevzky 2010). Mead, also known as honey wine, was made in Asia between 1700 and 1100 BC, as well as in Europe and the Middle East. "In Egypt, Babylon, Rome, and China, people produced wine from grapes and beer from malted barley. In South America, people produced *chicha* from grains or fruits, mainly maize; while in North America, people made *octli* (now known as "pulque") from agave, a type of cactus" (Alba-Lois and Segal-Kischinevzky 2010).

Prior to Louis Pasteur, a French chemist, it was unknown what process or reactions were responsible for the formation of fermented beverages. In 1860, Pasteur discovered that live yeast cells actively convert glucose into ethanol. Additionally, Pasteur "demonstrated that only microorganisms are capable of converting sugars into alcohol from grape juice," and that this process occurs under anaerobic conditions (Alba-Lois and Segal-Kischinevzky 2010).

In the past decade, hundreds of strains of *Saccharomyces cerevisiae* have been isolated from around the world for the purposes of taxonomic studies. These studies identify a high degree of genetic diversity in the strains of yeast used in different regions of the world; many strains of yeast are localized, or present only in certain regions of the world (Table 2) (Cornuet, Karst and Legras 2007). Thus, when a baker claims the yeast used in their fermentation is exclusively found in one micro-region of the world, this may be plausible; however, genetic diversity must be verified through genetic tests and taxonomic study.

Technical Advisory Panel (TAP) Reports were written on baker's, brewer's, autolysate, nutritional, and smoked yeast in 1995. These TAP Reports were presented later that year for review by the National Organic Standards Board (NOSB) as they considered these materials for inclusion in the National Organic

Program (NOP) National List, which was made official in 2002. In 2006, the NOSB was petitioned to move yeast from §205.605(a) for nonagricultural substances to §205.606 for agricultural substances on the basis that yeast is an agricultural product derived from nature, and remains unaltered under laboratory settings. The petition suggested that processors be required to use organic yeast if commercially available (Marroquin International Organic Commodities Services, Inc. 2006; Siegel 2010). This petition was resubmitted in 2010, and the NOSB formally recommended to the NOP for "organic forms, when commercially available, be required for human consumption." Yeast was not recommended to be moved to §205.606 "due to concerns of the livestock industry and the debatable agricultural nature of yeast" (National Organic Standards Board 2010). The NOP later completed rulemaking resulting in the current annotation.

**Organic Foods Production Act, USDA Final Rule:**

Yeast and microorganisms do not appear in OFPA.

The National List includes under §205.605(a) for nonsynthetics allowed, "Yeast—When used as food or a fermentation agent in products labeled as 'organic,' yeast must be organic if its end use is for human consumption; nonorganic yeast may be used when organic yeast is not commercially available. Growth on petrochemical substrate and sulfite waste liquor is prohibited. For smoked yeast, nonsynthetic smoke flavoring process must be documented."

Genetic engineering or modification are excluded methods as per §205.2 (Electronic Code of Federal Regulations: Part 205-National Organic Program 2013). §205.105(e) indicates that excluded methods are prohibited in organic products.

**International**

Yeast is separately listed as an allowed substance in Canada and the European Union. No separate listing for yeast is included in the regulatory bodies for the CODEX Alimentarius, Japan, and IFOAM. All regulatory bodies have regulations either restricting or explicitly prohibiting the inclusion of genetically modified yeast and/or microorganisms for use in organic production. Specific regulatory information from each international ruling body is provided in the corresponding sections below.

*Canadian General Standards Board*
Table 6.4, titled "Non-organic Ingredients Not Classified as Food Additives" of the Canadian Permitted Substance List states that only nonsynthetic yeast is allowed in organic handling. The types of yeast include "autolysate, bakers' (may contain lecithin, obtained without the use of bleaches and organic solvents), brewers', nutritional, and smoked. Non-synthetic smoke flavouring process shall be documented. Growth on petrochemical substrate and sulfite waste liquor are prohibited" (Canadian General Standards Board 2011).

*CODEX Alimentarius*
Yeast in the CODEX Alimentarius does not appear separately as it does in the USDA organic regulations. Under Additives and Processing Aids, "probiotics, microorganisms and enzymes are allowed." GL 32-1999 section 3.4 states that "any preparations of microorganisms and enzymes normally used as processing aids in food processing" are permitted for use "with the exception of genetically engineered/modified organisms and enzymes derived from genetically engineered/modified organisms." Additionally, Table 3.1 (Food additives, including carriers) makes a reference to calcium sulfate with the restriction of "cakes & biscuits/soy bean products/bakers yeast".

*European Economic Community Council*
Article 20 allows for the labeling of organically produced yeast as organic, and states that "only organically produced substrates are to be used for the production of organic yeast and organic yeast should not be present in organic food or feed together with non-organic yeast" (The Council of the European Union 2008). On July 10, 2008, the panel for organic yeast recommended "temporarily allowing 5% non-organic yeast

extract for the production of organic yeast, until organic yeast extract is available, as additional substrate for the production of organic yeast as a source of nitrogen, phosphor, vitamins and minerals....The availability of organic yeast extract or autolysate will be re-examined by December 31, 2013 with a view to withdrawing this provision" (The Council of the European Union 2008). Substances listed in Annex VIII, Section C, and materials referenced in Article 27(1)(b) and (e) of this Regulation are allowed to be used in the production of yeast (The Council of the European Union 2008).

Article 9 states that genetically modified organisms (GMOs) and products produced by or from GMOs are prohibited in organic production (The Council of the European Union 2007). Article 19 states that "only additives, processing aids, flavorings, water, salt, preparations of micro-organisms and enzymes...may be used, and only in so far as they have been authorized for use in organic production in accordance with Article 21" (The Council of the European Union 2007). An additional restriction is that "products and substances referred to in Article 19(2)(b) are to be found in nature and may have undergone only mechanical, physical, biological, enzymatic or microbial processes, except where such products and substances from such sources are not available in sufficient quantities or qualities on the market" (The Council of the European Union 2007).

*Japan Agricultural Standard (JAS)*
The JAS Standard for Organic Processed Food does not specifically identify the allowance for yeast, in Table 1: Food Additives of the Japanese Agricultural Standard for Organic Processed Foods (Japanese Agricultural Standard for Organic Processed Foods (Notification No. 1606) 2005). However, the standard includes the following language that indicates that microorganisms, including yeasts, are allowed: "Only physical method or method using biological function (except for those produced by the recombinant DNA technology; hereafter the same) shall be used for the manufacturing or processing." (Article 4: Criteria of Production Methods – Management concerning manufacturing, processing, packaging, storage and other processes). The term "biological function" indicates the permitted use of microorganisms such as yeasts. Additionally, there are references to yeast in this and other documents, such as the allowance of calcium sulfate as a "coagulating agent for confectionary, processed bean products, or bread yeast."

Questions and Answers on the Japanese Agricultural Standards for Organic Plants and Organic Processed Foods Q21-15 state "since culturing materials for microorganisms are not considered to be direct ingredients of organic processed foods, in cases where it is unavoidable, it is permissible to use microorganisms cultured with: materials other than organic plants, organic processed foods and organic livestock products" and "materials modified with recombinant DNA technology. However, should culturing materials for microorganisms be used in significant quantity (5% or more) in the manufacturing of processed foods, and remain there without being removed, said materials will be viewed as ingredients." In summary, yeast must not be genetically modified, and when organic ingredients are commercially unavailable nonorganic ingredients are allowed for use with the restrictions stated above.

*International Federation of Organic Agriculture Movements (IFOAM)*
Yeast is permitted in IFOAM per Section 7.2.5 which states that "preparations of micro-organisms and enzymes commonly used in food processing may be used, with the exception of genetically engineered micro-organisms and their products. Cultures that are prepared or multiplied in-house shall comply with the requirements for the organic production of microorganisms." Section 7.2.6 states that "yeast shall be included in the percentage calculations of organic ingredients by 2013." Additionally, the section titled "Preparations of Micro-organisms and Enzymes for use in food processing" states, "these may be used as ingredient or processing aids with approval from the control body: organic certified micro-organisms, preparations of micro-organisms…." Yeast, as a microorganism, is likely interpreted in this document to contain the restrictions of microorganisms, and ruling bodies may place additional restrictions where necessary.

## 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)).**

While yeast is a nonsynthetic material, there may be ancillary ingredients added that are not on the National List. Ancillary ingredients are discussed in detail in the Combinations of a Substance section above.

*Baker's and brewer's yeast*
In general, yeast is grown using raw materials which "are usually agricultural, forestry and food waste by-products. There are two types of raw materials depending on the grown organism: conventional materials (e.g. starch, molasses, distiller's wash, whey, fruit and vegetable wastes, wood, straw, etc.) and unconventional ones (e.g. petroleum by-products, natural gas, ethanol and methanol)" (Bekatorou, Psarianos and Koutinas 2006). The terms "conventional" and "unconventional" are not to be mistaken for terms used in the organic industry such as "organic" and "conventional." The vitamins biotin, inositol, pantothenic acid, and thiamine are necessary for yeast growth, requiring growth substrate to contain these nutrients. Additional nutrients required for yeast growth are listed in Table 6, and alternate material sources in Table 7.

**Table 6.** Nutrients required for yeast growth and example material sources (Safriet 1994; Organic Materials Review Institute (OMRI) 2013)

| Raw Nutrients | Material Source |
|---|---|
| Nitrogen | Ammonium salts (Ammonium sulfate**) |
| | Aqueous ammonia |
| | Anhydrous ammonia |
| Potassium | Molasses |
| Calcium | Molasses |
| Phosphate | Phosphoric acid |
| | Phosphate salts (monoammonium phosphate**) |
| Magnesium | Magnesium salts (Magnesium sulfate**, magnesium chloride** |
| Iron | Ferrous sulfate** |
| Zinc | Zinc sulfate |
| Copper | Copper sulfate** |
| Manganese | Manganese sulfate** |
| Molybdenum | Trace |
| Biotin | Cane molasses* |
| Inositol | Molasses |
| Pantothenic acid | Molasses |
| Thiamine | |
| Sugar | Corn grits |
| | Raisins |
| | Cane molasses |
| | Beet molasses |
| Amino acids** | |
| Pyridoxine** | |

| | |
|---|---|
| Water** | |
| | |

* Beet molasses is deficient in biotin **OMRI Products Database

**Table 7.** Growth media feedstock for the "production of yeast using alternative, low cost waste by-products of the food and agricultural industries" (Bekatorou, Psarianos and Koutinas 2006)

| **Microorganism** | **Raw material** |
|---|---|
| *Rhodotorula rubra, Candida tropicalis, C. utilis, C. boidinii, Trichosporon cutaneum* | Salad oil manufacturing wastewater |
| *Candida arborea* | Rice straw hydrolysate |
| *Candida halophila, Rhodotorula glutinis* | Glutamate fermentation wastewater |
| *Saccharomyces cerevisiae* | Extracts of cabbage, watermelon, green salads and tropical fruits |
| *Candida utilis* | Defatted rice polishing |
| *Candida versatilis, Kluyveromyces lactis, Kluyveromyces marxianus* | Whey |
| *Candida utilis, Pichia stipitis, Kluyveromyces marxianus, Saccharomyces cerevisiae* | Waste Chinese cabbage |
| *Candida utilis* | Apple pomace |
| *Saccharomyces cerevisiae* | Virgin grape marc |
| *Saccharomyces sp., Pichia sp., Rhodotorula sp., Candida sp., Kluyveromyces sp.* and *Trichospora sp.* | Lettuce brine |
| *Candida langeronii* | Cane bagasse hemicellulosic hydrolyzate |
| *Torulopsis cremoris, Candida utilis, Kluyveromyces fragilis* | Whey |
| *Pichia guilliermondii* | Waste brine from kimchi production |
| *Geotrichum candidum* | Orange peel |
| *Candida, Rhodotorula, Leucosporidium* | Prawn shell waste |
| *Hansenula sp.* | Sugar beet stillage |
| *Candida utilis* | Pineapple cannery effluent |
| *Saccharomyces cerevisiae* | Waste date products |
| *Saccharomyces cerevisiae* | Hydrolyzed waste cassava |
| *Saccharomyces cerevisiae, Torula utilis, Candida lipolytica* | Deproteinized leaf juices of turnip, mustard, radish and cauliflower |
| *Saccharomyces cerevisiae* | Shrimp shell waste |
| *Candida krusei, Saccharomyces sp.* | Sorghum hydrolysate |
| *Candida rugosa* | Sugar beet stillage |
| *Kluyveromyces fragilis* | Cheese whey |
| *Cellulomonas flavigena, Xanthomonas sp.* | Sugarcane bagasse pith |
| *Candida spp. (utilis, tropicalis, parapsilosis and solani)* | Molasses and sugar beet pulp |
| *Kluyveromyces, Candida, Schizosaccharomyces sp.* | Jerusalem artichoke |
| *Pichia pinus* | Mango waste or methanol |

Molasses is "the most widely used substrate for baker's yeast production" (Bekatorou, Psarianos and Koutinas 2006). Alternatively, whey is used exclusively as a primary substrate by specific strains of yeast in the genus *Kluyveromyces* and *Candida* because "*Saccharomyces cerevisiae* lacks the enzyme β-galactosidase and lactose permease. *Kluyveromyces marxianus* is the only strain used for biomass production from whey on a commercial scale" (Bekatorou, Psarianos and Koutinas 2006).

At a commercial production facility, yeast is in general "fermented, harvested, concentrated and/or dried and packaged" (Bekatorou, Psarianos and Koutinas 2006). The fermentation process takes place within bioreactors operated at 30 °C and pH of 4.5-5, where the growth medium is present, and in an aerobic environment. Fermenting under anaerobic conditions would alternatively use sugars to produce ethanol and $CO_2$ instead of cell growth, resulting in low yeast yields (Safriet 1994). Many foreign bacteria and other microorganisms grow under the same conditions as yeast. These can outcompete the desired yeast, hence the requirement for sterile/aseptic procedures to be maintained throughout the production of yeast (Safriet 1994).

Initially, cells from a pure yeast culture are grown on a suitably adjusted mixture of molasses in the laboratory, and the produced biomass is transferred aseptically into the bioreactors (Bekatorou, Psarianos and Koutinas 2006). Initial growth of yeast is accomplished in a laboratory, where a small batch of pure yeast is blended together with molasses malt and grown for 2 to 4 days under sterile conditions. The entire mixture is then added to the first fermenter, where the yeast grows for an additional 13 to 24 hours during what is called the "pure culture stage" (Safriet 1994). This yeast mixture is then transferred to a second fermenter for stock fermentation to allow for larger scale growth with a matured yeast culture. This stage involves regular feeding cycles and continuous aeration. Upon completion, the mixture is centrifuged to separate the stock yeast for the final fermentation stage prior to commercial fermentation, which involve further increased aeration, molasses, and other nutrients which are fed incrementally, which lasts from "11 to 15 hours." After the final feeding, "the liquid is aerated for an additional 0.5 to 1.5 hours to permit further maturing of the yeast, making it more stable for refrigerated storage" (Safriet 1994).

After the yeast has reached a sufficient mass[4], the cells are separated via centrifugation and "further concentrated by a filter press or rotary vacuum filter" to produce a filter cake. "The filter cake is then blended with small amounts of water, emulsifiers, and cutting oils (e.g. soybean or cottonseed oil) to form the end product...which is cooled to below 8 °C" (Safriet 1994) as fresh baker's yeast, "or thermalized and dried to form various types of dry yeast. The dried yeast is packed under vacuum or nitrogen gas atmosphere" (Bekatorou, Psarianos and Koutinas 2006).

*Nutritional yeast*

The production process for nutritional yeast differs only slightly from that of baker's and brewer's yeast. "Pure strains of *Saccharomyces cerevisiae* are grown on mixtures of cane and beet molasses. After the fermentation process is complete, the yeast is harvested, washed, pasteurized, dried, and packaged" (Adeduro and Snyder 2003). Pasteurization of yeast is common only for the production of nutritional yeast, and was not identified as a manufacturing step for other types of yeast.

*Smoked yeast*

Smoked flavoring for products such as yeast is produced both synthetically and nonsynthetically. Smoked flavoring can be produced nonsynthetically by directly exposing yeast to smoke produced from the burning of wood. Alternatively, smoke comes from a smoke generator, making use of fans, which allows the smoke to come into contact with water (40-140 °F), forming a liquid smoke with an acidity between 3 and 8% that can be applied to food products (Clifford 1963).

Smoked flavoring can be produced synthetically by passing "smoke produced by a generator through a trap containing water (Freshel 1954)," filtering out undesirable ingredients, and extracting the smoke flavoring with an organic solvent such as diethyl ether or ethanol for direct use on ingredients such as

[4] The quantity of yeast produced depends on the number of fermentations the yeast goes through. Typically, this is 15,000 to 100,000 kg when a 4 fermentation stage process is used, and 7,500 to 50,000 kg for a 2 stage fermentation process. Roughly "half of the yeast manufacturing facilities" in this study used the 4 stage fermentation process. (Safriet 1994)

yeast (Wasserman, Method for imparting hickory smoke color and flavor to dried yeast and other food powders 1971). The FDA has imposed regulations for artificial smoked flavors at 21 CFR, which states that "any pyroligneous acid and other artificial smoke flavors" cannot provide any statement or declaration that indicate food ingredients have been smoked, have a "true smoked flavor," or that by using artificial smoke flavors as a seasoning or flavor will "result in a smoked product or one having a true smoked flavor" (US FDA 2013).

*Yeast autolysate*

Two primary types of yeast autolysis processes are used: induced and natural (Alexandre and Guilloux-Benatier 2006).

Induced autolysis is generally performed in industry, as opposed to natural autolysis, and "can be induced by physical inductors (rise in temperature, alternate freezing and thawing, and osmotic pressure), chemical inductors (pH, detergents, and antibiotics), or biological inductors (aeration and starvation). The autolysis process can be very fast, from 48 h to 72 h, depending on the inducer" (Alexandre and Guilloux-Benatier 2006). Alternatively, natural autolysis is promoted by "using a mixture of killer and sensitive yeast for the secondary fermentation (of wine); under these conditions a rapid death of sensitive yeast cells occurs in the presence of killer strains" (Alexandre and Guilloux-Benatier 2006). Killer strains of yeast are further described under Evaluation Question #9.

The mechanism for autolysis results in the degradation of the cell, including the cell walls and intracellular structures. This process results in much of the cell becoming hydrolyzed, and water soluble. During the final stage of cell wall degradation, "the hydrolytic products are released when their molecular masses are low enough to cross pores in the cell wall." When the cell ruptures, or becomes permeable, nucleosides, nucleotides, amino acids, peptides, proteins, and lipids are released, while the cell wall breaks down into glucan, mannoprotein (Alexandre and Guilloux-Benatier 2006), and chitin (Kollar, et al. 1997).

**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.**

The primary method to produce yeast involves fermentative growth on nutrient rich media substrate (Suomalainen and Oura 1971) (OMRI 2013). The media substrate varies, and was previously outlined in detail in the manufacturing process section above. However, one such substrate with industrial use, and which is identified in the current USDA organic regulations, is sulfite waste liquor (Suomalainen and Oura 1971) (USDA National Organic Program 2013). However, the nutritive components of the waste liquor vary with the types of wood used in the paper making process, and the degree of cooking in the pulping process (Inskeep, et al. 1951).

Since yeast is produced through fermentation and separated from residual growth media and concentrated using mechanical or physical methods (i.e., filtration or centrifugation) (Borrows 1970; OMRI 2013), the manufacturing process can be considered a naturally occurring biological process, and yeast can be classified as nonsynthetic.

Smoked yeast is traditionally manufactured by passing smoke through dried yeast as it tumbles in a rotating drum (Wasserman, Method for imparting hickory smoke color and flavor to dried yeast and other food powders 1971). However, smoked yeast products are also manufactured using chemical processes that leave the final smoked yeast product containing synthetic materials. In this chemical process, a liquid smoke concentrate is added to a volatile solvent such as diethyl ether, which is then directly added to a dry yeast powder (Wasserman, Method for imparting hickory smoke color and flavor to dried yeast and other food powders 1971).

Yeast is currently listed as a nonagricultural substance at section 205.605 in the USDA organic regulations. However, discussion within the organic sector concerning the classification of yeast as agricultural or nonagricultural has a long history. A petition to reclassify yeast as agricultural was submitted in 2006 and again in 2010 (Marroquin International Organic Commodities Services, Inc. 2006; Siegel 2010). The NOP received official public comment starting as early as 2008 regarding the reclassification of yeast and the restructuring of the processing lists in general (Rulemaking Program Management Office 2008). The public comment discussion centered on the need to clarify the definition of agricultural and nonagricultural with the possibility of amending section 205.605(a) to include nonorganic materials, or, in other words, materials that are impossible to produce organically (Wyard 2008; Rosen and Zuck 2008). The NOP responded to a 2010 NOSB recommendation by finalizing Guidance Document 5014, which clarifies that yeast, even though it is classified as nonagricultural, can be certified organic; however, when used as a livestock feed ingredient is not required to carry certification (National Organic Program 2010). The NOP acted further on the NOSB recommendation in 2012 by updating the yeast listing at §205.605(a) to clarify that when yeast is used for human consumption, it must be certified organic when commercially available (USDA Agricultural Marketing Service 2012 ).

**<u>Evaluation Question #3:</u> 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)).**

Yeast is considered a nonsynthetic microorganism with exceptions where yeast which has been genetically modified. A TAP Review (1995) for yeast autolysate used in processing states that it can be either synthetic or nonsynthetic, depending on whether the growth medium is organic, and whether only physical separation methods are involved. According to the NOSB Materials Database, yeast autolysate "can be made from brewer's yeast, baker's yeast, alcohol-grown yeast or whey-grown yeast." Genetically modified strains of yeast are widely available and readily found in literature, including strains of *Saccharomyces cerevisiae* (Matsushika, Inoue and Sawayama 2009), *Candida utilis* (Ikushima, et al. 2009), and *Saccharomyces boulardii* (Rottiers, Vandenbroucke and Iserentant 2012), and strains which are considered GRAS.

**<u>Evaluation Question #4:</u> 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.**

Yeast is listed at 7 CFR §205.605(a) nonsynthetics allowed of the USDA organic regulations. Additionally, yeast is identified at 21 CFR §170.3(o)(17) as leavening agents. 21 CFR §101.22(3) defines yeast and yeast extract as natural flavors by stating "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…edible yeast…" The regulatory status of specific types of yeast are outlined in Tables 8-10.

**Table 8**. *Bakers yeast*

| Section in 21 CFR | Identity | Species/Substance |
|---|---|---|
| §172.896<br>Not GRAS | Dried yeasts | Dried yeast (Saccharomyces cerevisiae and Saccharomyces fragilis) and dried torula yeast (Candida utilis) |
| §172.381<br>Not GRAS | Vitamin D2 baker's yeast | Saccharomyces cerevisiae that has been exposed to ultraviolet light, producing vitamin $D_2$ through a photochemical reaction with endogeneous ergosterol, a steroid found in fungi. |

**Table 9.** *Yeast autolysate*

| Section in 21 CFR | Identity | Species/Substance |
|---|---|---|
| §184.1983 | Baker's yeast extract | Yeast autolysate. |

| | | |
|---|---|---|
| §172.325<br>Not GRAS | Baker's yeast protein | The insoluble proteins remaining after the cell walls of Saccharomyces cerevisiae have been ruptured, and whole cell walls and soluble materials have been removed. |

**Table 10**. *Selenium Yeast*

| Section in 21 CFR | Identity | Species/Substance |
|---|---|---|
| GRAS<br>GRN No. 260<br>GRN No. 353 | High-selenium yeast | Selenium yeast. Derived from Saccharomyces cerevisiae. |

*Brewers yeast*
No specific FDA regulatory citation for non-genetically modified strains.

*Nutritional yeast*
No specific FDA regulatory citation.

*Smoked yeast*
No specific FDA regulatory citation.

**<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)).**

The primary technical function of yeast in food production is in the fermentation of beer, wine, and spirits; leavening of bread, flavor, nutrition, and other health benefits such as probiotics. Yeast is not identified as a preservative in literature. However, byproducts of yeast, such as ethanol, are considered preservative agents due to their prevention of mold and microbial growth (Floros and Ozdemir 2004). Additionally, baker's yeast mannoprotein is present in the GRAS Notice Inventory for use "as a stabilizing agent in wines, at levels ranging from 50 to 400 milligrams per liter, to prevent tartaric acid precipitation" (GRN 284). Specific strains of yeast, such as those within *Saccharomyces cerevisiae,* produce toxins that are lethal to other strains of *Saccharomyces cerevisiae* and which inhibit contamination of a mature yeast culture (U.S. Environmental Protection Agency 1997).

**<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)).**

Yeast is used primarily to produce alcohol and leaven breads in organic production. However, in addition to these primary uses, yeast products such as yeast extracts, nutritional yeast, and smoked yeast products are used as flavor additives in processed products by supplying a savory umami[5] taste (Populin, et al. 2007; Nagodawithana 1992). Specific regulations allow for the use of autolyzed yeast in various canned vegetables (FDA 2013) and as a "flavoring agent and adjuvant (FDA 2000)" as defined at 21 CFR 170.3(o)(12) (FDA 2013).

Studies have also shown that certain yeast strains, the so called "red yeasts," can be used to impart coloring to foods and also to animal (trout, salmon, lobster and chicken egg yolks) pigmentation (Lyons, Jacques and Dawson 1993; Marova, Certik and Breierova 2012).

[5] Umami is identified as one of the five primary tastes and is imparted by glutamate, a non-essential amino acid, and ribonucleotides, including inosinate and guanylate (Unami Information Center website, 2013).

**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)).**

Nutritional benefits are provided by nutritional yeast, brewer's yeast, yeast extract and probiotic yeast products. All of these products are used as health supplements for a variety of different reasons, including as protein supplement, immunity enhancer, and for the control of diabetes (Moyad 2008; Rabinowitz, et al. 1983).

Nutritional yeast is inactivated yeast with no leavening power, has a high protein profile, and is rich in amino acids and B-vitamins (Red Star Yeast 2013). Some B vitamins are also present in brewer's yeast, but not in the amounts found in nutritional yeast. Brewer's yeast is, however, high in metals, including chromium which may have beneficial health effects (Offenbacher, Rinko and Pi-Sunyer 1985; Simonoff, et al. 1992). Yeast extract products that are used as flavoring agents, flavor enhancers, protein sources (6.1% total N) and binders are composed primarily of amino acids, peptides, proteins, carbohydrates, fats and salts (Food Chemicals Codex 2003). Yeast species, specifically *Saccharomyces boulardii,* are used for their probiotic properties (Czerucka, Piche and Rampal 2007). This yeast strain has been shown to have probiotic properties and can be used for its therapeutic effect for treatment of antibiotic-associated diarrhea and recurrent intestinal infections.

An additional, indirect benefit of yeast should be considered here when assessing its effects on the nutritional quality of foods. Fermentation, the digestive action of bacterial or fungal cells (yeast), can positively affect the levels of nutrients in food both by increasing phytase, a phosphatase enzyme which converts indigestible phosphorous into digestible forms (Türk, Carlsson and Sandberg 1996), and through a process called "pre-digestion," where hard to digest compounds are broken down into more available forms (Katz 2012).

**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)).**

Yeast or yeast byproducts are not included as part of the FDA's action levels of Mercury, Cadmium and Lead, as included in the FDA's booklet Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed (FDA 2000). According to the Toxic Substances Control Act (TSCA), *Saccharomyces cerevisiae* is exempt from reporting (EPA 2000). The TSCA inventory does not cover chemical substances addressed by other U.S. statutes such as foods and food additives.

The Food Chemicals Codex (FCC) lists threshold levels of lead (Pb) and mercury (Hg) in yeast autolysate, dried yeast and yeast extract as follows (Food Chemicals Codex 2003). Thresholds are reported as mg/kg, which is equal to ppm.

Yeast autolysate and Yeast Extract: Pb < 2 mg/kg (ppm) and Hg < 3 mg/kg (ppm)

Dried Yeast: Pb- < 1 mg/kg (ppm), no threshold for Hg

Yeasts are capable of bioaccumulation of metals which can be valuable (e.g. selenium and chromium yeast) but also may have potential toxicity concerns (Brady and Duncan 1994). The ability of yeast to act as a bioabsorbent has metal-sequestering implications where yeast biomass has successfully removed silver, gold, californium, cobalt, chromium, copper, nickel, lead, uranium, thorium and zinc from aqueous solution (Wang and Chen 2009). *Saccharomyces cerevisiae* has been shown to accumulate heavy metals such as cobalt and cadmium (Norris and Kelly 1977). Therefore, the growth media and water sources used for commercial yeast production can significantly impact the presence of heavy metals in yeast products.

**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)).**

The Final Risk Assessment, an environmental and human impact study performed by the EPA for *Saccharomyces cerevisiae* states that this organism is "ubiquitous in nature... and the only adverse effect to the environment noted in the literature is the presence of the 'killer toxins'…composed of proteins and glycoproteins" in a few strains of *Saccharomyces cerevisiae,* which is lethal to other strains of *Saccharomyces cerevisiae* (U.S. Environmental Protection Agency 1997). These particular strains have been infected by dsRNA viruses from the *Totiviridae* family, a type of mycovirus (J. and Breinig 2006).

In the manufacture of yeast, "volatile organic compound (VOC) emissions are generated as byproducts of the fermentation process. The two major VOCs emitted are ethanol and acetaldehyde" (Safriet 1994). Minor VOC's produced and released as part of the manufacturing process include "alcohols such as butanol, isopropyl alcohol, 2,3-butanediol, organic acids and acetates. Based on emission test data, approximately 80 to 90 percent of total VOC emissions is ethanol, and the remaining 10 to 20 percent consists of other alcohols and acetaldehyde" (Safriet 1994). Acetaldehyde is a hazardous air pollutant as defined under Section 112 of the *Clean Air Act* (U.S. EPA 2013).

The Final Risk Assessment for *Saccharomyces uvarum* states that it has been "isolated from such natural sites as honey, phyllosphere, on the surfaces and inside rotten fruit, and in fruit juice." Literature does not indicate that this species of yeast has a negative environmental impact, pathogenic attributes, or toxin production against flora or fauna. Therefore, the manufacture and use of *Saccharomyces uvarum* is unlikely to be harmful to the environment" (U.S. Environmental Protection Agency 1997).

While yeast itself may be considered a minimal risk material to both the environment and in use, the manufacturing process for yeast will have a negative environmental impact which can be mitigated with appropriate waste management (Adeduro and Snyder 2003). This is primarily due to the emission of acetaldehyde and ethanol, discussed in Evaluation Question #10.

**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)).**

Baker's yeast:
A Final Risk Assessment published by the U.S. Environmental Protection Agency (EPA) on *Saccharomyces cerevisiae* states that "*Saccharomyces* are frequently recovered from the stools and throats of normal healthy individuals. This indicates that humans are in constant contact with these yeasts. There are individuals who may ingest large quantities of *S. cerevisiae* every day as part of a 'health food' regimen." Due to its documented presence in the human gastrointestinal system, *S. cerevisiae* is not considered a pathogenic microorganism according to the EPA. However, rare cases of yeast infections have occurred in individuals with immunodeficiencies and those consuming antibiotics regularly for treatment. This is because antibiotics kill or weaken the natural flora present in biological systems (U.S. Environmental Protection Agency 1997). The Final Risk Assessment additionally states that "there have been no reports of isolates of *S. cerevisiae* that produce toxins against either humans or animals."

A Final Risk Assessment published by the U.S. EPA on *Saccharomyces uvarum* states that this species is used in the production of beer, wine, and ethanol and has "no reported incidences of adverse effects to humans." It additionally quotes an article by Stewart and Russell (1985) stating "no other group of microorganisms has been more intimately associated with the progress and wellbeing of the human race than *Saccharomyces cerevisiae* and its closely related species." Humans have been exposed to *S. uvarum* in production facilities and in research environments, and currently the "consumption of yeast (generic application) is a common source of vitamins…a history of significant exposure with incidence of disease in a nondebilitated condition contributes to a history of safe use." This Final Risk Assessment additionally states that "closely related species also have a history of extensive use without significant incidence of disease. The most intensely studied of those species closely related to *S. uvarum* is *S. cerevisiae*...There were no reports found in the literature that indicate that *S. uvarum* produces toxins to humans or animals."

High selenium yeast has also been identified as a possible anti-carcinogen (Finley, Davis and Feng 2000). Selenium (Se), an essential nutrient, is important to human biology as it is essential to enzymatic function (Rayman 2000).

As referenced previously in Evaluation Question #9, acetaldehyde is a byproduct of yeast production. "Acute (short term) and chronic (long term) inhalation exposure to acetaldehyde is associated with adverse health effects including irritation to the eyes, skin and respiratory tract. Acetaldehyde is also a potential developmental toxin and a probable human carcinogen" (Adeduro and Snyder 2003) (U.S. EPA 2012) . Though yeast itself generally has either no health impacts, or beneficial health impacts with the exception of individuals with weakened or compromised immune systems, the yeast production process does produce byproducts such as acetaldehyde and ethanol that are documented VOCs and are harmful to human health (U.S. Environmental Protection Agency 1997).

As mentioned previously, yeast autolysate contains naturally occurring monosodium glutamate (MSG). The Joint FAO/WHO Expert Committee on Food Additives (JECFA), composed of "independent scientists, drawn mainly from government or academic research institutes" performed a safety evaluation on MSG to determine any negative health impacts (Walker and Lupien 2000). JECFA concluded that "the total dietary intake of glutamates arising from their use at levels necessary to achieve the desired technological effect and from their acceptable background in food do not represent a hazard to health." The Scientific Committee for Food of the Commission of the European Communities (SCF) came to the same conclusion with its own study as the JECFA. The Federation of American Societies for Experimental Biology (FASEB), under contract with the FDA, characterized "MSG symptom complex" to include the following symptoms: "a burning sensation of the back of the neck, forearms and chest; facial pressure or tightness; chest pain; headache; nausea; upper body tingling and weakness; palpitation; numbness in the back of the neck, arms and back; bronchospasm (in asthmatics only); and drowsiness" (Walker and Lupien 2000). The FASEB report concluded that "although there was no scientifically verifiable evidence of adverse effects in most individuals exposed to high levels of MSG, there is sufficient documentation to indicate that there is a subgroup of presumably healthy individuals that responds, generally within 1 hour of exposure, with manifestations of the MSG symptom complex when exposed to an oral dose of MSG of 3 g *in the absence of food*" (Walker and Lupien 2000). Despite the symptoms appearing in the absence of food, it was noted that the testing method of using single doses and simple solutions could not accurately predict adverse reactions that may result from MSG present in food. The FDA interpreted these results, and determined MSG to be safe for consumption in general and that there was no evidence to indicate that MSG in food was linked to adverse reactions in the general population (Walker and Lupien 2000).

One yeast manufacturing plant identified by an EPA study pipes their ethanol waste to a water treatment plant for use as fuel in a boiler to reduce atmospheric pollution (Adeduro and Snyder 2003). In the atmosphere, ethanol undergoes a photochemical reaction forming a hydroxyl radical which has a half-life of 5 days (United States National Library of Medicine 2004).

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

Wild yeasts were traditionally employed in the fermentation of alcoholic beverages (Kunkee and Bisson 1993; Beech 1993). However, the reliance on wild yeast varieties has given way to more standardized yeast cultures to provide a more uniform fermentation and to meet health and safety concerns.

**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)).**

Natural (nonsynthetic) substances or products are available as alternatives to yeast products used as flavors as identified and discussed earlier in this report. Umami is identified as one of the five primary tastes, and is imparted by glutamate, a non-essential amino acid, and ribonucleotides, including inosinate and guanylate (Umami Information Center website, 2013). Both glutamate and ribonucleotides occur naturally in many foods such as tomato, potato, Chinese cabbage, mushroom, carrot, soybean and green tea (Ninomiya 1998, Jinap 2010). Extracts of these substances could possibly be used in place of yeast extract as a flavor enhancer. For example, a mushroom extract powder blended with palm oil and sprayed on a maltodextrin carrier is currently marketed as an umami rich flavor enhancer (Nikken Foods 2012). Other similar products that may be used in place of yeast extract include Chinese extract powder (Nikken Foods 2012) and seaweed powder (Nikken Foods 2012), among others.

For the purposes of leavening in baking, sodium carbonate and sodium bicarbonate are two natural (nonsynthetic) alternatives to yeast which are currently included in the USDA organic regulations at §205.605(a). As alkali materials, both substances are used in combination with other substances in recipes. These other substances include acidic ingredients such as citrus juice, vinegar or sour cream. Other alternatives to the use of yeast for leavening include chemical leaveners. These include chemicals such as ammonium carbonate and bicarbonate or baking powder (potassium acid tartrate) These substances are chemical leavening agents, as opposed to yeast as a biological leavening agent, and are allowed synthetic ingredients currently listed at §205.605(b) of the USDA organic regulations. The action of chemical leavening agents is much the same as leavening via yeast: to aerate the dough making it light and porous (Pyler 1973).

Literature has not shown any other nonsynthetic alternatives to yeast in the production of alcoholic beverages.

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

Organic agricultural alternatives including vegetable extracts such as mushroom extract powder, seaweed extract powder and cabbage extract powder could be used as alternatives to yeast extracts for flavoring. However, the commercial availability of certified organic extracts is unclear. While these extract types are high in glutamate and ribonucleotides (Ninomiya 1998) (Jinap 2010) they may not provide the correct flavor profile that is provided by yeast extracts.

Literature has not shown any organic agricultural alternatives to yeast used for the production of alcohol or used as a leavening agent.

As part of this Technical Report, OMRI asked Accredited Certification Agents (ACAs) about their experiences with certified organic yeast products. One ACA answered that they certify "yeast batch for ingredient in beer production" Many ACAs have clients using certified organic yeast, nonorganic yeast autolysate, and smoked yeast according to the annotation at 205.605(a). ACAs generally note that there is currently not certified organic yeast available in the form, quality and quantity needed.

ACAs freely responded to OMRI and it appears form and quality are the greatest factors in finding organic yeast. One ACA described the dialogue with their client over the use of organic yeast in the following way, "They (the client) usually start their search by asking their current supplier about organic sources which usually ends in 'there aren't any that we know of currently'." That ACA also identified that most of their clients acknowledge there is currently only one manufacturer of organic yeast but they do not have the quality or form that the client needs. ACAs recognize the difficulties in obtaining yeast in the form and quality needed. Specifically, some ACAs have received negative comments on quality noting that client needs such as a yeast with a long shelf life are not being met with the available organic yeasts.

## References


Ackerman, Emanuel, and Seymour Pomper. Preparation of active dry yeast. USA Patent US 3448010 A. June 3, 1969.

Adeduro, K., and E. Snyder. *ADOPT-A-MACT PROJECT: A Manual of Model Documents and Guidance to aid Compliance and Enforcement of the NUTRITIONAL YEAST MACT (40 CFR Part 63, Subpart CCCC).* Compliance Document for Auditors, Office of Radiation and Compliance Assurance AIR Division US EPA, 2003.

Alba-Lois, Luisa, and Claudia Segal-Kischinevzky. "Yeast Fermentation and the Making of Beer and Wine." *Nature Education*, 2010: 3(9):17.

Aleck, Robbins E., Schuldt Jr E. Henry, Seeley R. Dudley, Sidoti D. Robert, and William Sucher R. Yeast glycan and process of making same. United States Patent US3867554A. February 18, 1975.

Alexandre, Herve, and Michele Guilloux-Benatier. "Yeast autolysis in sparkling wine - a review." *Australian Journal of Grape and Wine Research*, 2006: 119-127.

Baron, Samuel. *Medical Microbiology. 4th edition.* Galveston: The University of Texas Medical Branch at Galveston, 1996.

Battikh, Houda, Amina Bakhrouf, and Emna Ammar. "Antimicrobial effect of Kombucha analogues." *LWT - Food Science and Technology*, 2012: 71-77.

Beech, Frederick W. *Yeasts in Cider Making.* Vol. 5, in *The Yeasts: Yeast Technology, 2nd edition*, by A.H. Rose, J.S. Harrison and ed, 169-213. London: Academic Press Limited, 1993.

Bekatorou, Argyro, Costas Psarianos, and Athanasios A. Koutinas. "Production of Food Grade Yeasts." *Food Technol. Biotechnol.*, 2006: 407-415.

Bennett, J.W. "Mycotechnology: The role of fungi in biotechnology." *Journal of Biotechnology*, 1998: 101-107.

Borrows, S. *Baker's Yeast.* Vol. 3, in *The Yeasts*, by A.H. Rose, J.S. Harrison and ed. London: The Academic Press , 1970.

Boulton, Chris, and David Quain. *Brewing Yeast and Fermentation.* Oxford: Blackwell Science, 2001.

Brady, D, and J.R. Duncan. "Bioaccumulation of metal cations by Saccharomyces cerevisiae." *Applied Microbiology and Biotechnology 41, no. 1*, 1994: 149-154.

Canadian General Standards Board. *Organic Production Systems Permitted Substances Lists CAN/CGSB-32.311-2006.* Regulation, Government of Canada, 2011.

Caplice, Elizabeth, and Gerald F. Fitzgerald. "Food fermentations: role of microorganisms in food production and preservation." *International Journal of Food Microbiology*, 1999: 131-149.

Cauvain, Stan, ed. *Bread making: improving quality.* . Cambridge: Woodhead Publishing , 2003.

Clifford, Maurice H. Aqueous smoke solution for use in foodstuffs and method of producing same. United States of America Patent US3106473 A. October 8, 1963.

Cornuet, J.-M., F. Karst, and J.-L. Legras. "Bread, beer and wine: Saccharomyces cerevisiae diversity reflects human history." *Molecular Ecology*, 2007: 2091-2102.

Czerucka, D., T. Piche, and P. Rampal. "Review article: yeast as probiotics - Saccharomyces boulardii." *Alimentary Pharmacology and Therapeutics*, 2007: 767-778.

Czeruka, D., T. Piche, and P. Rampal. "Review article: yeast as probiotics -Saccharomyces boulardii." *Alimentary Pharmacology & Therapeutics*, 2007: 767-778.

Diehl, John, T.P Lyons, and K.A. Jacques. "All in good taste: creating natural savory flavorings from yeast." *Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 20th Symposium: reimiganing the feed industry.* Lexington: Alltech UK, 2004. 257-263.

Dubey, R.C., D.K. Maheshwari, and R. Saravanamurthu. *Industrial Exploitation of Microorganisms.* New Delhi: I.K. International Publishing House Pvt. Ltd., 2010.

*Electronic Code of Federal Regulations: Part 205-National Organic Program.* 9 13, 2013. http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&SID=325a45a3a8b33d74ac16c037eb4c7b2b&rgn=div5&view=text&node=7:3.1.1.9.32&idno=7 (accessed 9 17, 2013).

EPA . "Yeast Extract Hydrolysate from Saccharomyces cerevisiae; Exemption from the Requirement of a Tolerance." *Federal Register Vol.69, No. 42.* Washington DC, March 03, 2004.

EPA. "Title 40-Protection of Environment." *40 CFR 725.420 - Recipient microorganisms.* Washington DC: US Government, July 1, 2000.

European Food Emulsifier Manufacturers' Association. *EFEMA index of food emulsifiers.* Industry Report, Brussels: EFEMA, 2013.

European Molecular Biology Laboratory: The European Bioinformatics Institute. *InterPro Protein sequence analysis & classification.* 2013. http://www.ebi.ac.uk/interpro/entry/IPR006215 (accessed November 1, 2013).

Evans, E. G. V., J. Heritage, and R. A. Killington. *Introductory microbiology.* New York: Cambridge University Press, 2000.

FAO/WHO. *Guidelines for the Evaluation of Probiotics in Food.* London Ontario, 2002.

FDA. "21CFR 172.896 Dried Yeasts." *TITLE 21--FOOD AND DRUGS CHAPTER I--FOOD AND DRUG ADMINISTRATION DEPARTMENT OF HEALTH AND HUMAN SERVICES SUBCHAPTER B--FOOD FOR HUMAN CONSUMPTION (CONTINUED) PART 172 -- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION* . Washington DC, June 01, 2013.

—. *FDA Home: GRAS Notice Inventory: GRN No. 260.* August 31, 2013. http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=260 (accessed November 20, 2013).

—. *FDA Home: GRAS Notice Inventory: GRN No. 353.* August 31, 2013. http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=353 (accessed November 20, 2013).

—. "Food Additive Status List." *Food Additives and Ingredients* . Silver Springs, Md: US FDA, March 21, 2013.

—. *GRAS Notice Inventory.* August 31, 2013. http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?filter=yeast&sortColumn=&rpt=grasListing (accessed November 25, 2013).

—. *Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed.* August 1, 2000. http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/chemicalcontaminantsmetalsnaturaltoxinspesticides/ucm077969.htm#merc (accessed November 26, 2013).

FDA. "Listing of Specific Substances Affirmed as GRAS Section 184.1983 - Bakers yeast extract. ." *DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE.* Washington, DC, 2000.

—. "Re: GRAS Notice No. GRN 000284." *Agency Response Letter GRAS Notice No. GRN 000284.* College Park, Md: Center for Food Safety and Applied Nutrition, US FDA, August 28, 2009.

—. "Re: GRAS Notice No. GRN 000422." *Agency Response Letter GRAS Notice No. GRN 000422.* College Park, Md: Center for Food Safety and Applied Nutrition, US FDA, August 31, 2012.

FDA. "Section 184.1983 - Bakers yeast extract." *Part 184 - DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE.* Washington DC, April 01, 2000.

FDA. "Subpart B--Requirements for Specific Standardized Canned Vegetables." *CHAPTER I--FOOD AND DRUG ADMINISTRATION DEPARTMENT OF HEALTH AND HUMAN SERVICES SUBCHAPTER B--FOOD FOR HUMAN CONSUMPTION* . Washington DC, June 01, 2013.

FDA. "TITLE 21--FOOD AND DRUGS CHAPTER I--FOOD AND DRUG ADMINISTRATION DEPARTMENT OF HEALTH AND HUMAN SERVICES SUBCHAPTER B--FOOD FOR HUMAN CONSUMPTION ." *Part 170 FOOD ADDITIVES.* Washington DC, April 01, 2013.

Fermentis . *Springer Oenologie* . Technical Sheet, Marcq en baroeul: Fermentis-Division of S.I. Lesaffre, 2012.

Finley, John W. , Cindy D. Davis, and Yi Feng. "Selenium from high selenium broccoli protects rats from colon cancer." *The Journal of nutrition 130, no. 9*, 2000: 2384-2389.

Floros, John D., and Murat Ozdemir. "Active Food Packaging Technologies." *Critical Reviews in Food Science and Nutrition*, 2004: 185-193.

*Food Chemicals Codex.* Washington, DC: The National Academies Press, 2003.

Freshel, C. Method of preparing smoked yeast products. United States of America Patent US2677614A. May 4, 1954.

Gaynor, Paulette. "How U.S. FDA's GRAS Notification Program Works." *Food Safety Magazine*, December 1, 2005.

Gregory, JF, 3rd. "Chemical and nutritional aspects of folate research: analytical procedures, methods." *Adv Food Nutr Res 33,* 1989: 1-101.

Guadalupe, Zenaida, Leticia Martinez, and Belen Ayestaran. "Yeast Mannoproteins in Red Winemaking: Effect on Polysaccharide, Polyphenolic, and Color Composition." *American Journal of Enology and Viticulture*, 2010: 191-200.

Hambridge K, Michael. "Chromium nutrition in man." *The American Journal of Clinical Nutrition 27. no. 5*, 1974: 505-514.

Hammond, John R.M. *Brewer's Yeast.* Vol. 5, in *The Yeasts: Yeast technology, Vol. 5*, by Anthony H. Rose and J Stewart Harrison, 7-69. London: Academic Press Limited, 1993.

Hansen, Egon B. "Microorganisms with technologically beneficial use." *Summilk IDF World Dairy Summit 2011.* Parma, 2011.

Hassan, H. "Antioxidant and Immunostimulating Activities of Yeast (Saccharomyces cerevisiae) Autolysates." *World Applied Sciences Journal*, 2011: 1110-1119.

Hatoum, Rima, Steve Labrie, and Ismail Fliss. *Antimicrobial and Probiotic Properties of Yeast: From Fundamental to Novel Applications.* Lausanne: Front. Microbio., 2012.

Ikushima, Shigehito, Toshio Fujii, Osamu Kobayashi, Satoshi Yoshida, and Aruto Yoshida. "Genetic Engineering of Candida utilis Yeast for Efficient Production of L-Lactic Acid." *Bioscience, Biotechnology, and Biochemistry*, 2009: 1818-1824.

Ingraham, John L. *March of the Microbes: Sighting the Unseen.* Cambridge, MA: Belknap of Harvard UP, 2010.

Inskeep, G.C., A.J. Wiley, J.M. Holderby, and L.P. Hughes. "Food Yeast from Sulfite Waste LIquor." *Industrial and Engineering Chemistry 43, no. 8* , 1951: 1702-1711.

J., Scgnuttm Manfred, and Frank Breinig. "Yeast viral killer toxins: lethality and self-protection." *Nature*, 2006: 212-221.

*Japanese Agricultural Standard for Organic Processed Foods (Notification No. 1606).* Regulation, Ministry of Agriculture, Forestry and Fisheries, 2005.

Jinap, S and Parvaneh Hajeb. "Glutamate. Its applications in food and contribution to health." *Appetite 55, no. 1*, 2010: 1-10.

Kamel, Fouad H. "Preparation of Organic Selenocystine Using Locally Isolated Bread Yeast." *Journal of Life Sciences*, 2013: 57-62.

Katz, Sandor Ellix. *The Art of Fermentation: An in-Depth Explration of Essential Concepts and Processes from around the World.* . White River Junction: Chelsea Green Publishing, 2012.

Kollar, Roman, et al. "Architecture of the Yeast Cell Wall." *The Journal of Biological Chemistry*, 1997: 17762-17775.

Kunkee, Ralph E., and Linda F. Bisson. "Wine-making Yeasts." In *The yeasts: Yeast Technology, Second Edition*, by A.H. Rose, J.S. Harrison and eds., 69-127. London: Academic Press, 1993.

Kurtzman, Cletus, J.W. Fell, and Teun Boekhout. *The Yeasts: A Taxonomic Study, Volume 1.* Burlington: Elsevier B.V., 2011.

Lallemand . *Danstar Product Data Sheet Listings.* Lallemand. January 1, 2010. http://www.danstaryeast.com/products/datasheets (accessed November 14, 2013).

Lallemand Inc. *Baking Update: Instant Yeast Volume 2, Number 9.* Info sheet, Montreal: Lallemand Inc., 1998.

Lallemand Inc. *The Baking Update: Yeast Production Volume 1, Number 9.* Industry Update, Montreal: Lallemand Inc., 1996.

Lodolo, Elizabeth J. , Johan L.F. Kock, Barry C. Axcell, and Martin Brooks. "The yeast Saccharomyces cerevisiae– the main character in beer brewing." *FEMS Yeast Research* 8, no. 7 (October 2008): 1018-1036.

Lyons, T.P. , K.A. Jacques, and K.A. Dawson. *Miscellaneous Products from Yeast.* Vol. 5, in *The Yeasts: Yeast Technology, 2nd Edition*, by Anthony H. Rose, J. Stuart Harrison and eds., 1-6. London: Academic Press Inc., 1993.

Mapari, Sameer A., Kristan F. Nielsen, Thomas O. Larsen, Jens C. Frisvad, Anne S. Meyer, and Ulf Thrane. "Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants." *Current Opinion in Biotechnology*, 2005: 231-238.

Marova, Ivana, Milan Certik, and Emilia Breierova. "Production of enriched biomass by carotenogenic yeasts– Application of whole-cell yeast biomass to production of pigments and other lipid compounds." In *Remote Sensing of Biomass - Principles and Applications*, by Temilola Fatoyinbo and ed., 345-384. Rijeka: InTech, 2012.

Marroquin International Organic Commodities Services, Inc. "National Organic Program: Petitioned Substances Database." *Petitioned Substances Database.* August 9, 2006. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5057569 (accessed November 20, 2013).

Matsushika, Akinori, Hiroyuki Inoue, and Shigeki Sawayama. "Ethanol production from xylose in engineered saccharomyces cerevisiae strains: current state and perspectives." *Applied Microbiology and Biotechnology*, 2009: 37-53.

Ministry of Agriculture, Forestry and Fisheries. *Japanese Agricultural Standard for Organic Plants.* Regulation, Ministry of Agriculture, Forestry and Fisheries, 2012.

Mo, Haizhen, Yang Zhu, and Zongmao Chen. "Microbial fermented tea - a potential source of natural food preservatives." *Trends in Food Science & Technology*, 2008: 124-130.

Mooradian, Arshag D, Mark Failla, Byron Hoogwerf, Melinda Maryniuk, and Judith Wylie-Rosett. "Selected vitamins and minerals in diabetes." *Diabetes care 17, no. 5*, 1994: 464-479.

Moslehi-Jenabian, Saloomeh, Line Lindegaard, and Lene Jespersen. "Beneficial effects of probiotic and food borne yeasts on human health." *Nutrients 2, no. 4*, 2010: 449-473.

Moyad, M.A. . ""Brewer's/baker's yeast (Saccharomyces cerevisiae) and preventive medicine: Part II." *Urologic nursing 28, no. 1*, 2008: 73.

Nagodawithana, T. "Yeast-derived flavors and flavor enhancers and their probable mode of action." *Food Technology, 11*, 1992: 138-144.

National Organic Program. *Guidance Document Certification of Yeast.* Washington DC, March 02, 2010.

National Organic Standards Board. "National Organic Program: Petitioned Substances Database." *Petitioned Substances Database.* October 28, 2010. http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateJ&page=NOPNationalList (accessed November 1, 2013).

Nikken Foods. *Champignon Mushroom Extract Powder Technical Data Sheet.* Technical Data Sheet, St. Louis: Nikken Foods, 2012.

Nikken Foods. *Roasted chinese cabbage extract powder.* Technical Data Sheet, St. Louis: Niken Foods, 2012.

Nikken Foods. *Seaweed Powder.* Technical Data Sheet, St. Louis: Nikken Foods, 2012.

Ninomiya, Kumiko. "Natrual Occurence." *Food Reviews International 14, no. 2-3*, 1998: 177-211.

Norris, P.R. , and D.P. Kelly. "Accumulation of Cadmium and Cobalt by Saccharomyces cerevisiae." *Journal of General Microbiology, Vol. 99*, 1977: 317-324.

Northern Arizona University. *Yeast diagram.* 2008. http://www4.nau.edu/biology/bio205/handouts.htm (accessed November 22, 2013).

Offenbacher, E.G., C.J. Rinko, and F.X. Pi-Sunyer. "he effects of inorganic chromium and brewer's yeast on glucose tolerance, plasma lipids, and plasma chromium in elderly subjects." *The American journal of clinical nutrition 42*, 1985: 454-461.

Office of Air Quality Planning and Standards. *Compilation of Air Pollutant Emission Factors.* Stationary Point and Area Sources, Research Triangle Park: U.S. Environmental Protection Agency, 1995.

OMRI. "ACA Survey ." *OMRI Certifier Survey - Waxes and Food Coatings, Yeast.* Eugene: OMRI, November 23, 2013.

OMRI. *OMRI Products Database.* Eugene, Or, November 19, 2013.

Organic Materials Review Institute (OMRI). *OMRI Product Database.* Eugene, November 19, 2013.

Populin, Tiziana, Sabrina Moret, Simone Truant, and Lanfranco S. Conte. "A survey on the presence of free glutamic acid in foodstuffs, with and without added monosodium glutamate ." *Food Chemistry 104* (Elsevier), 2007: 1712-1717.

Pyler, Ernst J. . *Baking.* Kansas City: Sosland Publishing Company, 1973.

Rabinowitz, Michael B., Harvey C. Gonick, Seymour R. Levin, and Mayer B. Davidson. "Effects of chromium and yeast supplements on carbohydrate and lipid metabolism in diabetic men." *Diabetes Care 6, no. 4*, 1983: 319-327.

Rakin, Marica, Maja Vukasinovic, and Milan Maksimovic. "Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate." *Food chemistry*, 2007: 599-602.

Rayman, Margaret P. "The Importance of Selenium to Human Health." *The Lancet 35, no. 9225* , 2000: 233-241.

Red Star Yeast. *Original Red Star Yeast: Nutritional Yeast.* January 1, 2013. http://www.redstaryeast.com/science-yeast/types-yeast/nutritional-yeast (accessed November 25, 2013).

Rose, Anthony H., and Govindaswamy Vijayalakshmi. *Baker's Yeast.* Vol. 5, in *The Yeasts: Yeast Technology, Second Edition*, by A.H. Rose and J.S. ed. Harrison, 357-397. London: Academic Press Limited, 1993.

Rosen, Emily Brown, and Leslie Zuck. *Docket ID: AMS-TM-08-0021.* Spring Mills, May 5, 2008.

Rottiers, Pieter, Klass Vandenbroucke, and Dirk Iserentant. Use of Recombinant Yeast Strain Producing an Anti-Inflammatory Compound in the Manufacture of a Medicament to Treat Colitis. European Union Patent EP1931762 (B1). December 5, 2012.

Rulemaking Program Management Office. "search results." *regulations.gov.* May 2008, 2008. http://www.regulations.gov/#!searchResults;rpp=25;po=0;s=Docket%252BNo.%252BAMS-TM-08-0021 (accessed November 19, 2013).

Safriet, Dallas. *Emission Factor Documentation for AP-42 Section 9.13.4 Yeast Production.* Final Report , Research Triangle Park: U.S. EPA, 1994.

Sazawal, Sunil, Girish Hiremath, Usha Dhingra, Pooja Malik, Saikat Deb, and Robert E Black. "Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials." *The Lancet infectious diseases 6, no. 6* , 2006: 374-382.

Siegel, Richard D. "National Organic Program: Petitioned Substances Database." *Petitioned Substances Database.* May 25, 2010. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5085213 (accessed November 1, 2013).

Simonoff, Monique, Dennis Shapcott, Said Alameddine, Marie Thèrèse Sutterdub, and Gabriel Simonoff. "The isolation of glucose tolerance factors from brewer's yeast and their relation to chromium." *Biological trace element research 32, no. 1-3*, 1992: 25-38.

Skogerson, Lawrence E. Method for the Production of Chromium Yeast. United States Patent US4348483. September 7, 1982.

Soccol, Carlos R., Ashok Pandey, and Christian Larroche. *Fermentation Processes Engineering in the Food Industry.* Taylor & Francis Group, LLC, 2013.

Stearns, Diane M. "Is chromium a trace essential metal?" *Biofactors*, 2000: 149-162.

Suomalainen, Heikki, and Erikki Oura. *Yeast Nutrition and Solute Uptake.* Vol. 2, in *The Yeasts: Physiology and Biochemistry of Yeasts*, by Anthony H. Rose, J.S. Harrison and ed., 3-74. London: Acedemic Press Inc. Ltd, 1971.

Sweeney, M.J., S White, and D.W. Dobson. "Mycotoxins in agriculture and food safety." *Irish Journal of Agricultural and Food Research* , 2000: 235-244.

The Council of the European Union. "European Commission: Legislation: Commission Regulation (EC) No 1254/2008 of 15 December 2008." *Commission Regulation (EC) No 1254/2008 of 15 December 2008.* December 15, 2008. http://ec.europa.eu/agriculture/organic/eu-policy/legislation_en (accessed November 8, 2013).

—. "European Commission: Legislation: Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91." *Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91.* June 28, 2007. http://ec.europa.eu/agriculture/organic/eu-policy/legislation_en (accessed November 8, 2013).

Türk, Maria , Nils-Gunnar Carlsson, and Anne-Sofie Sandberg. "Reduction in the levels of phytate during wholemeal bread making; effect of yeast and wheat phytases." *Journal of Cereal Sciences 23, no. 3,* 1996: 257-264.

U.S. Environmental Protection Agency. *Saccharomyces cerevisiae Final Risk Assessment.* February 1997. http://www.epa.gov/biotech_rule/pubs/fra/fra002.htm (accessed September 26, 2013).

—. *Saccharomyces uvarum Final Risk Assessment.* February 1997. http://www.epa.gov/oppt/biotech/pubs/fra/fra010.htm (accessed September 26, 2013).

U.S. EPA. *EPA Home: Air & Radiation: Assessing Outdoor Air Near Schools:Monitored Pollutants.* October 17, 2012. http://www.epa.gov/schoolair/pollutants.html (accessed November 12, 2013).

—. *EPA Home: Air& Radiation: TTN Web - Technology Transfer Network: Air Toxics Web site: Original list of hazardous air pollutants.* August 8, 2013. http://www.epa.gov/ttn/atw/orig189.html (accessed November 12, 2013).

U.S. FDA. *GRAS.* 8 21, 2013. http://www.fda.gov/food/ingredientspackaginglabeling/gras/microorganismsmicrobialderivedingredients/default.htm (accessed 9 17, 2013).

United States National Library of Medicine. *Substances List: Ethanol.* 2004. http://webwiser.nlm.nih.gov/getSubstanceData.do?substanceID=18&displaySubstanceName=Absolute%20Ethanol&STCCID=&UNNAID=&selectedDataMenuItemID=62 (accessed November 12, 2013).

US FDA. *CFR - Code of Federal Regulations Title 21.* April 1, 2013. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.22 (accessed November 15, 2013).

USA FDA. *Title 21 Chapter 1 Subchapter B Section 172.590-- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION.* 2013.

—. *Title 21, Chapter 1, Subchapter B, Part 172.896 FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION.* 2013.

—. *Title 21, Chapter 1, Subchapter B, Part 172.898- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION.* 2013.

USDA Agricultural Marketing Service . "Federal Registry Volume 77, Number 8." *Document Number AMS-NOP-09-0074; NOP-09-01PR RIN 0581-AC96.* Washington, DC, January 12, 2012 .

USDA Agricultural Marketing Service. "List of Certified USDA organic operations." *2012 List of certified USDA organic operations.* Washington, D.C. : USDA, January 02, 2013.

USDA. *Commerical Item Description: Yeast, Baker's (active).* CID, Washington DC: USDA , 2001.

USDA National Organic Program. *The National List of Allowed and Prohibited Substances.* Washington DC, November 19, 2013.

Vieira, Elsa, Olivia Pinho, Isabel Ferreira, and Tiago Brandão. "Saccharomyces cerevisiae brewing biomass as a promising source of nucleotides for flavour enhancers production." *IJUP '12 5th Meeting of Young Researchers of University of Porto.* Porto: University of Porto, 2012. 3000-3007.

Walker, Ronald, and John R. Lupien. "The Safety Evaluation of Monosodium Glutamate." *The Journal of Nutrition,* 2000: 1049-1052.

Wang, Jianlong, and Can Chen. "Biosorbents for heavy metals removal and their future." *Biotechnology advances 27, no. 2* , 2009: 195-226.

Wasserman, Aaron E. . Method for imparting hickory smoke color and flavor to dried yeast and other food powders. USA Patent US 3615728 A. October 26, 1971.

Wasserman, Aaron E. Method for imparting hickory smoke color and flavor to dried yeast and other food powders. USA Patent US3615728A. October 26, 1971.

Watson, Dennis C. *Yeasts in Distilled Alcoholic-Beverage Production.* Vol. 5, in *The Yeast: Yeast Technology, Second Edition,* by A.H. Rose, J.S. Harrison and ed., 215-244. London: Academic Press Limited, 1993.

Williams, A.N., and K.M. Woessner. "Monosodium glutamate 'allergy': menace or myth?" *Clinical & Experimental Allergy 39, no. 5,* 2009: 640-646.

Wyard, Gwendolyn. *Re: Clarifications of Definitions (Docket No. AMS-TM-08-0021).* Salem, May 5, 2008.

Yoshida, Yoshiko. "Umami taste and traditional seasonings." *Food Reviews International 14, no 2-3* , 1998: 213-246.