

§ 173.160

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be safely used in the production of cheese in accordance with the following prescribed conditions:

(a) Milk-clotting enzyme is derived from one of the following organisms by a pure-culture fermentation process:

(1) *Endothia parasitica* classified as follows: Class, Ascomycetes; order, Sphaeriales; family, Diaporthaceae; genus, *Endothia*; species, *parasitica*.

(2) *Bacillus cereus* classified as follows: Class, Schizomycetes; order, Eubacteriales; family, Bacillaceae; genus, *Bacillus*; species, *cereus* (Frankland and Frankland).

(3) *Mucor pusillus* Lindt classified as follows: Class, Phycomycetes; subclass, Zygomycetes; order, Mucorales; family, Mucoraceae; genus, *Mucor*; species, *pusillus*; variety, *Lindt*.

(4) *Mucor miehei* Cooney et Emerson classified as follows: Class, Phycomycetes; subclass, Zygomycetes; order, Mucorales; family, Mucoraceae; genus, *Mucor*; species, *miehei*; variety, *Cooney et Emerson*.

(5) *Aspergillus oryzae* modified by recombinant deoxyribonucleic (DNA) techniques to contain the gene coding for aspartic proteinase from *Rhizomucor miehei* var. *Cooney et Emerson* as defined in paragraph (a)(4) of this section, and classified as follows: Class, Blastodeuteromycetes (Hyphomycetes); order, Phialiales (Moniliales); genus, *Aspergillus*; species *oryzae*.

(b) The strains of organism identified in paragraph (a) of this section are nonpathogenic and nontoxic in man or other animals.

(c) The additive is produced by a process that completely removes the generating organism from the milk-clotting enzyme product.

(d) The additive is used in an amount not in excess of the minimum required to produce its intended effect in the production of those cheeses for which it is permitted by standards of identity established pursuant to section 401 of the Act.

[42 FR 14526, Mar. 15, 1977; 42 FR 56728, Oct. 28, 1977, as amended at 62 FR 59284, Nov. 3, 1997]

§ 173.160 *Candida guilliermondii*.

The food additive *Candida guilliermondii* may be safely used as the

organism for fermentation production of citric acid in accordance with the following conditions:

(a) The food additive is the enzyme system of the viable organism *Candida guilliermondii* and its concomitant metabolites produced during the fermentation process.

(b)(1) The nonpathogenic and nontoxicogenic organism descending from strain, American Type Culture Collection (ATCC) No. 20474,¹ is classified as follows:

Class: Deuteromycetes.
Order: Moniliales.
Family: Cryptococcaceae.
Genus: *Candida*.
Species: *guilliermondii*.
Variety: *guilliermondii*.

(2) The taxonomic characteristics of the reference culture strain ATCC No. 20474 agree in the essentials with the standard description for *Candida guilliermondii* variety *guilliermondii* listed in “The Yeasts—A Taxonomic Study,” 2d Ed. (1970), by Jacomina Lodder, which is incorporated by reference. Copies are available from the Center for Food Safety and Applied Nutrition (HFS-200), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

(c)(1) The additive is used or intended for use as a pure culture in the fermentation process for the production of citric acid using an acceptable aqueous carbohydrate substrate.

(2) The organism *Candida guilliermondii* is made nonviable and is completely removed from the citric acid during the recovery and purification process.

(d) The additive is so used that the citric acid produced conforms to the specifications of the “Food Chemicals Codex,” 3d Ed. (1981), under “Citric acid,” pp. 86–87, which is incorporated

¹Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

by reference. Copies may be obtained from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

[42 FR 14526, Mar. 15, 1977, as amended at 47 FR 11838, Mar. 19, 1982; 49 FR 10106, Mar. 19, 1984; 54 FR 24897, June 12, 1989]

§ 173.165 *Candida lipolytica*.

The food additive *Candida lipolytica* may be safely used as the organism for fermentation production of citric acid in accordance with the following conditions:

(a) The food additive is the enzyme system of the organism *Candida lipolytica* and its concomitant metabolites produced during the fermentation process.

(b)(1) The nonpathogenic organism is classified as follows:

Class: Deuteromycetes.
Order: Moniliales.
Family: Cryptococcaceae.
Genus: *Candida*.
Species: *lipolytica*.

(2) The taxonomic characteristics of the culture agree in essential with the standard description for *Candida lipolytica* variety *lipolytica* listed in "The Yeasts—A Taxonomic Study," 2d Ed. (1970), by Jacomina Lodder, which is incorporated by reference. Copies are available from the Center for Food Safety and Applied Nutrition (HFS-200), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

(c) The additive is used or intended for use as a pure culture in the fermentation process for the production of citric acid from purified normal alkanes.

(d) The additive is so used that the citric acid produced conforms to the

specifications of the "Food Chemicals Codex," 3d Ed. (1981), pp. 86-87, which is incorporated by reference. Copies may be obtained from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html. The additive meets the following ultraviolet absorbance limits when subjected to the analytical procedure described in this paragraph:

Ultraviolet absorbance per centimeter path length	Maximum
280 to 289 millimicrons	0.25
290 to 299 millimicrons	0.20
300 to 359 millimicrons	0.13
360 to 400 millimicrons	0.03

ANALYTICAL PROCEDURE FOR CITRIC ACID

GENERAL INSTRUCTIONS

Because of the sensitivity of the test, the possibility of errors arising from contamination is great. It is of the greatest importance that all glassware be scrupulously cleaned to remove all organic matter such as oil, grease, detergent residues, etc. Examine all glassware including stoppers and stopcocks, under ultraviolet light to detect any residual fluorescent contamination. As a precautionary measure it is recommended practice to rinse all glassware with purified isooctane immediately before use. No grease is to be used on stopcocks or joints. Great care to avoid contamination of citric acid samples in handling is essential to assure absence of any extraneous material arising from inadequate packaging. Because some of the polynuclear hydrocarbons sought in this test are very susceptible to photo-oxidation, the entire procedure is to be carried out under subdued light.

APPARATUS

1. Aluminum foil, oil free.
2. Separatory funnels, 500-milliliter capacity, equipped with tetrafluoroethylene polymer stopcocks.
3. Chromatographic tubes: (a) 80-millimeter ID x 900-millimeter length equipped with tetrafluoroethylene polymer stopcock and coarse fritted disk; (b) 18-millimeter ID x 300-millimeter length equipped with tetrafluoroethylene polymer stopcock.
4. Rotary vacuum evaporator, Buchi or equivalent.