Food and Agriculture Organization of the United Nations

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CITRIC ACID ESTERS OF MONO- AND DIGLYCERIDES OF FATTY ACIDS (CITREM)

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1. Summary

This Chemical and Technical Assessment summarizes data and information on citric acid esters of mono- and diglycerides of fatty acids (CITREM, INS No. 472c) submitted to JECFA by the sponsors, in November 2013, November 2017 and November 2018. CITREM is used as an emulsifier, flour treatment agent, sequestrant and stabilizer and is currently permitted for use in a wide range of foods. Its addition is limited by good manufacturing practice in most categories among those authorized.

CITREM consists of mixed citric acid esters of mono- and diglycerides made from edible fatty acids with glycerol. It may be partially or wholly neutralized with sodium hydroxide or potassium hydroxide of by using sodium, potassium or calcium salts of weak acids such as acetic, lactic propionic or carbonic acids. It is a fine, off-white powder.

CITREM (and related organic acid esters of mono- and diglycerides) was evaluated at its ninth (1966), tenth (1967) and seventeenth (1973) meetings (JECFA, 1966, JECFA, 1967, JECFA, 1974). An acceptable daily intake (ADI) "not specified" was allocated for CITREM (and related organic acid esters) at the seventeenth meeting. Specifications for CITREM were prepared at the 35th meeting (1989), published in FNP 49 (1990) and FNP 52 (1992). Specifications for metallic contaminants and arsenic were revised at the 61st JECFA (2003), 79th JECFA (2014), 82nd JECFA (2016) and 86th JECFA (2018) where the specifications were made tentative, requiring a replacement validated method for the obsolete packed column gas chromatographic method for the determination of total citric acid in its specifications monograph. A new package of information was received on new validated methods for the determination of total glycerol and total citric acid. Therefore, CITREM was included in the agenda of the 87th JECFA (2019), new specifications were prepared, and the tentative status was removed.

2. Description

2.1 Nature of the product

Citric acid esters of mono- and diglycerides are obtained from esterification of monoglycerides of fatty acids and citric acid. It can also be neutralized using sodium and potassium hydroxides. After crystallization, it is marketed as powders or pellets. Other synonyms for the citric acid esters of mono- and diglycerides of fatty acids are citroglycerides, mono- and diglycerides of fatty acids esterified with citric acid, CITREM, CAEM, CITREM. INS No. 472c; CAS# 97593-31-2.; E 472c.

2.2 History of use

CITREM is approved in most areas of the world (e.g. European Union, United States of America, Japan and Australia). The Committee has previously evaluated CITREM (and related organic acid esters of mono- and diglycerides) at its ninth (1966), tenth (1967) and seventeenth (1973) meetings (JECFA, 1966, JECFA, 1967, JECFA, 1974b). An acceptable daily intake (ADI) “not specified” was allocated for CITREM (and related organic acid esters) at the seventeenth meeting. JECFA has based its safety evaluation on biochemical and metabolic studies, which demonstrated that those substances are completely hydrolyzed in the gastrointestinal tract to citric or acetic or lactic acid, glycerol, and fatty acids, and that the metabolism of bound organic acids is not different than that of the free ones.

The use of CITREM as an emulsifier has a history of safe use in a wide variety of food products across Europe and USA. In Europe, the safety of CITREM for use in infant products and foods for special medical purposes for infants has previously been evaluated by the Scientific Committee for Food and has a history of safe use since its addition to the food additive provisions in 1995.

3. Manufacturing

3.1 Manufacturing principle

CITREM /INS 472c is obtained by esterification of glycerol with citric acid and edible fatty acids or by reaction of a mixture of mono- and diglycerides (MG and DG) of edible fatty acids with citric acid; it consists of mixed esters of citric acid and edible fatty acids with glycerol; may contain minor proportions of free fatty acids, free glycerol, free citric acid and mono- and diglycerides. Esterification is carried out at elevated temperatures (125-155 ºC) and water is removed under reduced pressure. The reaction progresses until a predetermined grade of esterification is reached. Then the product is cooled and packed; high melting forms can be spray cooled or pelletized. CITREM may be wholly or partially neutralized with food grade sodium, potassium or calcium salts of weak acids such as acetic, lactic, propionic or carbonic acids. The salts can be mixed into the CITREM as a solid or in aqueous solution. Addition is performed under conditions where CITREM at temperatures exceeding its melting point. The neutralization takes place under stirring and in some cases at reduced pressure to avoid oxygen and to remove water and other volatiles formed during salt addition. Neutralization leaves the CITREM as a salt while the neutralizing salt is in the free acid form. After the neutralization, the emulsifier may undergo a suitable separation process (such as drying) to remove water and other volatiles.

4. Chemical characterization

4.1 Composition and properties

CITREM consists of mixed esters of citric acid and edible fatty acids with glycerol; may contain minor proportions of free fatty acids, glycerol, citric acid and mono- and diglycerides; may be wholly or partially neutralized with sodium or potassium salts. The MG and DG present in CITREM may include either one or two edible fatty acids with chain lengths most commonly ranging from C12:0 to C18:0. In general, these are mainly the saturated fatty acids: palmitic acid (C16:0) and stearic acid (C18:0). CITREM may also contain lower concentrations of other fatty acids such as myristic acid (C14:0), oleic acid (C18:1), linoleic acid (C18:2) and arachidic acid (C20:4). The amount of the citric acid in the ester is typically 13%-25%.

The chemical structural formula of CITREM, resembles natural triglyceride lipids with a core glycerol backbone esterified at positions sn-1, sn-2, and sn-3. As indicated in Figure 1, at least one substituent at R_1, R_2 or R_3 is a citric acid moiety, one is a fatty acid moiety and the remainder may be citric acid, a fatty acid or hydrogen. For other organic acid esters the overall structure is identical with the exception that the citric acid moiety (R_1, R_2 or R_3) is replaced by acetic acid or lactic acid. An example of the chemical structure of CITREM is shown in Figure 2.

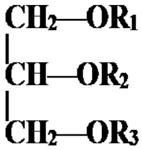

Figure 1. Chemical structural formula of CITREM and other organic acid esters of mono- and diglycerides of fatty acids

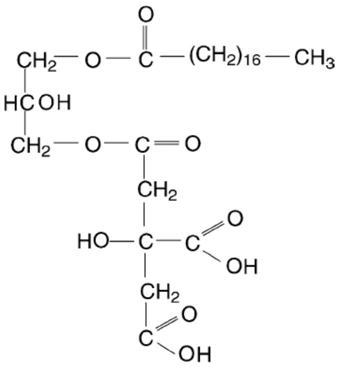

Figure 2. Example chemical structure of CITREM (Krog, 1997)

4.2 Physico-chemical properties

CITREM is insoluble in cold water, dispersible in hot water, soluble in oils and fats and insoluble in cold ethanol. Above its melting point, the emulsifier will hydrate and form viscous emulsions in water. Depending on the pH and the salts present in the system, the emulsions may be stable on cooling. The ester easily hydrolyses in presence of alkali or acid resulting in free citric acid, fatty acids and glycerol. The rate of the hydrolysis of the ester is dependent on the time and temperature. Enzymatic hydrolysis of the citric acid esters of mono- and diglycerides with lipases and esterases has also been reported. The ester exhibits significant interfacial properties resulting from the presence of a polar water-soluble moiety as well as a lipophilic portion on the same molecule.

The physical form of CITREM at room temperature is dependent on the fatty acid profile of the monoglycerides or diglycerides esterified with citric acid. A predominance of saturated fatty acids will yield high melting point CITREM, whereas a higher ratio of unsaturated fatty acids results in a liquid form of CITREM. In the latter case, the product is normally protected from autoxidation by addition of a suitable food grade antioxidant.

4.3 Possible impurities

The most important impurities are lead, cadmium and arsenic. Lead is limited to not more than 2 mg/kg according to JECFA specifications prepared at the 61st JECFA (2003), 79th JECFA (2014), 82nd JECFA (2016) 86th JECFA (2018) and modified in 87th JECFA (2019). At its 82nd meeting (2016), the Committee evaluated lead content for CITREM use in infant formula and in formula for special medical purposes and was revised at its 87th JECFA (2019) limiting lead levels to not more than 0.5 mg/kg). Sulphated ash limits were modified, establishing limits to not more than 0.5% for non-neutralized products, not more than 3% for partially neutralized products and not more than 10% for wholly neutralized products.

4.4 Analytical methods

The specifications monograph of CITREM cites general tests included in the FAO Combined Compendium of Specifications (FAO JECFA Monographs 1, vol. 4, 2006) and specific tests for the determination of glycerol, citric acid and fatty acids. (FAO JECFA Monographs 23, 2019). Total glycerol is determined by HPLC (Vol 4) after hydrolysis with KOH using a system equipped with a refractive index detector, autosampler and column thermostat; Aquasil C18 (250 mm x 4.6 mm x 5 µm) column or equivalent; a glycerol standard >99.5% and phosphoric acid 85%. After hydrolysis, the sample is diluted with phosphoric acid and 500 µl of the aqueous phase removed and filtered for HPLC analysis. The mobile phase consisted of 6.8 g KH_2PO_4 in 5 ml phosphoric acid made up to 1 litre in a volumetric flask. Other parameters are column and RI detector temperature (35 ºC), flow rate (1.0 ml/min) and injection volume (10 µl). Calculation is made by the following equation:

Total glycerol, % w/w = (CU (mg/ml) x 22 (ml) x 100) / W

where

W is the weight of sample, mg
CU is the concentration of glycerol determined from the curve (mg/ml)

Total citric acid is determined by HPLC after hydrolysis using a system equipped with an ultraviolet detector, autosampler and column thermostat; Synergi 4 µm Hydro RP 80 A (4.6 mm x 250 mm) or equivalent. Citric acid is used as standard (>99.5%) to construct a concentration curve. A methanolic extract of the sample is prepared and hydrolyzed for 2 h at 110 ºC with methanolic KOH 0.5 mol/l. After cooling, 20 ml of dilute phosphoric acid is added (5 ml phosphoric acid in 1 litre of water). 500 µl of the aqueous phase is filtered through a 0.45 µm membrane filter. The mobile phase consisted of 6.8 g KH_2PO_4 in 5 ml phosphoric acid made up to 1 l in a volumetric flask. Other parameters are column temperature (25 ºC), UV detector at 205 nm, flow rate 1.0 ml/min and injection volume (20 µl). The calculation is made by the following equation:

Total citric acid, % w/w= (CU (mg/ml) x 22 (ml) x 100%) / W

where

W is the weight of the sample, mg
CU is the concentration of the citric acid determined from the curve (mg/ml)

5. Functional uses

5.1 Technological function

CITREM is used as food emulsifier. The main food applications are margarines, shortenings, chocolate, dry yeast and powdered food (dispersant). Neutralized CITREM is used in sauces, dressings and meat products. CITREM is also used in various food applications like margarine production, where fine water droplets in the water in oil emulsion improve the frying properties of the margarine. Other food applications include special emulsions for bakery use, viscosity control in chocolate manufacturing, and meat emulsions. CITREM acts as a sequestrant, binding heavy metals, a property used in

combination with antioxidants to prevent the catalytic effect of heavy metals in the oxidation of fats and oils. CITREM is also used in powdered infant formula, follow on formula and in infant food for special medical purposes, formula manufactured with amino acids and hydrolyzed proteins and in energy dense ready-to-feed liquid infant formula. Formulations manufactured with amino acids and hydrolyzed proteins have different hydrophobic/hydrophilic characteristics and lower emulsifying capacity than products based on whole proteins. CITREM improves the stability and organoleptic properties of products containing (partially) hydrolysed proteins, peptides or amino acids. Emulsifiers are a technological requirement for these formulas to ensure both palatability and stability of the formula after reconstitution.

CITREM is an effective emulsifier of oil in water emulsions. CITREM is a stable non-ionic substance that is able to withstand harsh processing conditions such as spray drying and ultra-heat treatment. Its resistance to ionic interactions makes it suitable for use in products containing minerals and trace elements, such as infant formula. Furthermore, the presence of ions such as minerals and trace elements can also impact on the overall stability and palatability of the product.

The fat component of an infant formula has considerable impact on the integrity of an emulsion. CITREM has been found to be effective in infant formula that incorporate several oils to achieve the desired fatty acid profile, particularly for those formulas containing high levels of saturated fatty acids which would otherwise be difficult to emulsify. Infant formula high in medium chain triglycerides (MCT) are used in several medical conditions, such as intestinal lymphangectasia, chylothorax, long chain fatty acid oxidation disorders (these products contain upwards of 90% fatty acids as MCT) and liver disease. In these products, it is not the protein source (which may be whole protein) which gives rise to poor emulsion stability, but the combination of unusual fats and oils required to achieve the desired fatty acid profile. Also, some specialized infant formula do not contain protein in any form, and the use of emulsifiers with a superior emulsifying capacity is required to produce a stable and palatable reconstituted product. In addition, specialized infant formula are often fed through very narrow-bore nasogastric tubes and it is important that the products remain stable over prolonged periods. Emulsion stability reduces the risk of separation of fat and sedimentation of insoluble particles which may block feeding tubes. For specialized infant formula taken orally, palatability and product acceptance are clinically important, especially for infants over six months of age. An improved emulsion stability enhances the organoleptic properties and palatability of the product.

CITREM produces a stable, creamy-coloured emulsion, with less phase separation than formula containing a combination of lecithin, DATEM and mono- and di-acyl glycerols. The improved emulsion integrity enhances the stability of the reconstituted formula and gives better organoleptic properties than the formula. Taste and mouthfeel are important components of compliance and effectiveness of dietary management.

Emulsifier use in specialized infant formulas is constrained by the physical requirements of the product or the medical use. Gums, thickening agents and suspending agents (e.g. guar gum, alginates, alginic acid) are inappropriate in certain delivery systems and carrageenan use is contraindicated in gastrointestinal disease. Modified starches, containing small amounts of protein, are not suitable for use in hypoallergenic infant formula used in the dietary management of allergies and food protein intolerance.

5.2 Food categories and use levels

As per the General Standard for Food Additives (GSFA) of the Codex Alimentarius Commission, CITREM (INS No. 472c) is presently permitted for use in a wide range of foods as an emulsifier, flour treatment agent, sequestrant and stabilizer (GSFA, 2017) (see link for the food categories and maximum use levels). With the exception of complementary foods for infants and young children and infant formula FSMP in which CITREM can be added at levels up to 5 000 and 9 000 mg/kg, respectively, and of vegetable oils and fats and lard, tallow, fish oil, and other animal fats where it can be added at

levels up to 100 mg/kg, in all other food categories, its use is only limited by good manufacturing practice.

6. Reactions and fate in food

CITREM is stable in dry and cool conditions, but changes are observed at elevated temperatures and humidity. During storage at 66 ºC for 15 days, humidity allowed saponification and an increase in acid value and free glycerol was observed. However, when the emulsifier is in a solution in palm oil, it was found to be stable. The presence of the hydrophilic moiety (the citric acid and glycerol groups) as well as the lipophilic fatty acyl chain(s) in the same molecule permits the mixed ester to reduce interfacial tension between different phases of food systems.

The non-neutralized form of CITREM has a hydrophilic-lipophilic balance (HLB value) that can vary from 6 to 9. Emulsifiers with HLB values less than 10 are more hydrophobic and better suited for water in oil emulsions. Other factors such as the type and number of fatty acids, the amount of citric acid and the pH can also have an influence in the final food application (Palsgaard, 2011). CITREM, in a system of water and vegetable oil (e.g. soybean oil), reduce the surface tension. The acid moieties of the CITREM will interact with hydrophilic groups of food constituents, forming electrophilic or hydrogen bonds which will help stabilize interfaces within a complex food, such as an emulsion. This property makes CITREM widely used as an emulsifier to make small droplet size possible and stable. The thermodynamic characteristics of CITREM in an aqueous medium is related to its concentration, pH and temperature. The interaction of CITREM micelles in aqueous solution with proteins depends on the pH of the solution. The hydrophobicity is more pronounced at pH 5.5 than at pH above a protein's isoelectric point, leading to direct hydrophobic attraction between the non-polar regions of the protein and the hydrocarbon tails of CITREM.

CITREM in the monoglyceride form has two charged groups, especially at pH 7.2, facilitating electrostatic attraction and hydrogen bonding with proteins. As a consequence of the interaction of CITREM and proteins, the hydrophobic parts of both molecules are hidden in the interior of the mixed micelles formed, and the hydrophilic groups are directed towards the aqueous medium. Thus, the greater the protein association, the more the hydrophilic surface of the proteins is formed (Il'in et al., 2005).

Starches and derivatives also interact with proteins to form complexes with emulsifiers and affect starch gelatinization (Akuzawa et al., 1995). Polysaccharides, such as maltodextrins, can interact with CITREM and protein through hydrogen bonding, forming a ternary complex (maltodextrin + CITREM + protein) that also influences the functional properties of the protein (Anokhina et al., 2005).

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