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GRN 000681

November 15, 2016

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, Maryland 20740

Subject: Notice of a GRAS Exclusion for Isomaltulose Syrup (Dried)

Dear Sir/Madam:

In accord with 21 C.F.R. part 170, subpart E, Evonik Creavis GmbH (Evonik) hereby submits the enclosed notice that the use of isomaltulose syrup (dried) as a sucrose substitute in foods and beverages is excluded from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

Sincerely,

(b) (6)

Ricardo Carvajal

RC/sas
Enclosure

GRAS NOTICE FOR ISOMALTULOSE SYRUP (DRIED)
SUBMITTED BY EVONIK CREAVIS GMBH

Part 1 – Signed statements and certification

(1) Applicability of 21 C.F.R. part 170, subpart E

We submit this GRAS notice in accordance with 21 C.F.R. part 170, subpart E.

(2) Name and address of the notifier

Evonik Creavis GmbH
Paul-Baumann-Straße 1
45772 Marl, Germany

(3) Name of the notified substance

Isomaltulose syrup (dried)

(4) Applicable conditions of use of the notified substance

(a) Foods in which the substance is to be used

The substance is to be used in foods and beverages in which sucrose is used as an ingredient or is directly added by the consumer.

(b) Levels of use in such foods

Because the substance is used as a substitute for sucrose, it is used at the same levels as sucrose.

(c) Purpose for which the substance is used

The substance is used as a sucrose substitute.

(d) Description of the population expected to consume the substance

The population expected to consume the substance consists of members of the general population who consume at least one of the products described above.

(5) Basis for the GRAS determination

The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with section 170.30(a) and (b).

(6) Exclusion from premarket approval

The notified substance is not subject to the premarket approval requirements of the FDC Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

(7) Availability of data and information

If FDA asks to see the data and information that are the basis for our conclusion of GRAS status either during or after FDA's evaluation of our notice, we will agree to make the data and information available to FDA. Further, upon FDA's request, we will allow the Agency to review and copy the data and information during customary business hours at the above address, and will provide FDA with a complete copy of the data and information either in an electronic format that is accessible for the Agency's evaluation or on paper.

(8) Applicability of FOIA exemptions

None of the data and information in Parts 2 through 7 of our GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

(9) Certification

We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

(b) (6)

Name: Frank Hellmers
Title: Dipl.-Ing.

Date: 11.11.2016

Please address correspondence to:

Ricardo Carvajal
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Part 2 – Identity, method of manufacture, specifications, and physical or technical effect

(1) Identity of the notified substance

(a) Chemical name

Isomaltulose; Palatinose; 6-O-α-D-glucopyranosyl-D-fructofuranose

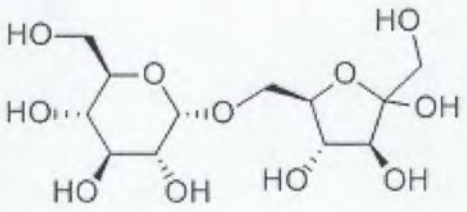
(b) Chemical Abstracts Service (CAS) Registry Number

13718-94-0

(c) Empirical formula

$C_{12}H_{22}O_{11} \cdot H_2O$

(d) Structural formula



(e) Characteristic properties

Isomaltulose is a reducing disaccharide consisting of a glucose and a fructose moiety linked by an alpha-1,6-glucosidic bond. Isomaltulose has similar food-technological properties as sucrose, except for its sweetness, which is about half that of sucrose (Südzucker, 2005), and its solubility in water, which also is about half that of sucrose at 50°C and even less at lower temperatures (Südzucker, 2005; i.e., GRN 000184 at pp. 9 and 10).

(f) Any known toxicants that could be in the source

The source material for the production of isomaltulose is food-grade sucrose. There are no known toxicants in the source.

(2) Description of method of manufacture

Evonik's isomaltulose syrup (dried) is produced by isomerizing food-grade sucrose with immobilized cells of *Protaminobacter rubrum* CBS 574.77 (see Annex 1 for a summary of the production process). This strain is used commercially for producing isomaltulose from sucrose as described, for example, in GRAS Notice GRN 000184 (FDA, 2006), in an application to the Commission of the European Union for the approval of isomaltulose as a novel food (Cargill, 2003) and in the final assessment report of Food Standards Australia New Zealand (FSANZ) of isomaltulose as a novel food (FSANZ, 2007).[1]

The difference between the subject product of this GRAS notice and the isomaltulose product considered in GRAS Notice GRN 000184 and authorized as a novel food in the European Union (Commission Decisions 2005/457/EC and 2005/581/EC) and in Australia/NZ (Australia New Zealand Food Standards Code – Standard 1.5.1 – Novel Foods) is mainly in the down-stream processing of the isomaltulose-containing solution that is obtained after the *Protaminobacter rubrum* catalyzed isomerization of sucrose. The down-stream processing that is applied by Evonik results in an isomaltulose syrup which has a somewhat lower isomaltulose content (≥ 80% on dry matter basis) than the isomaltulose product that is the subject of GRN 000184 (≥ 98%) and that is specified in FCC IX (Annex 2).

The starting material for Evonik´s isomaltulose syrup (dried) is food-grade sucrose. In a single enzyme-catalyzed reaction, sucrose is converted to isomaltulose. Cells of *Protaminobacter rubrum* strain CBS 574.77 that are immobilized in a matrix of calcium alginate and that have lost their viability during the immobilization process are used in this step as described earlier (Rose & Kunz, 2002, de Oliva-Neto & Menão, 2009).[2]

The enzyme of the immobilized cells of *Protaminobacter rubrum* that catalyzes the isomerization of sucrose to isomaltulose is a well characterized sucrose isomerase (EC 5.4.99.11) (McAllister et al., 1990; Ravaud et al., 2006; Rhimi et al., 2008; Ravaud et al., 2009).[3]

The use of an immobilized cell system (non-viable microbial cells immobilized in a calcium alginate gel bead) is a common industrial practice (Wojcik, 2002). For example,

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1 For a summary of the currently applied production methods of isomaltulose, see also Varzakas & Labropoulos, 2012 and Mu et al., 2014.

2 "*Protaminobacter rubrum*" is a still widely used denomination of this microorganism which is fact is "*Serratia plymuthica*" according to current taxonomic principles.

3 Cf. http://www.uniprot.org/uniprot/D0VX20.

it is used for the production of D-psicose from fructose as described in GRAS Notice GRN 000400. Calcium alginate, which is affirmed as GRAS for specified uses in foods as a stabilizer and thickener (21 CFR § 184.1187), is insoluble under both acidic and alkaline conditions. Residues of alginate in the isomaltulose syrup are therefore not expected and in fact do not occur (aqueous solutions of isomaltulose syrup are clear and transparent).[4]

The preparation of the immobilized, not viable *Protaminobacter rubrum* biomass is described in Annex 1.

The eluent is heat-treated for sterilization and then passed at 70°C through ion-exchange columns charged with cation and anion exchange resins that are appropriate for use in food production. The resultant demineralized solution is concentrated by evaporation and dried under reduced pressure and elevated temperature to yield a dry, white or colorless, crystalline powder as isomaltulose syrup (dried) (for specifications see Annex 3).

(3) Specifications for food-grade material

The Evonik specifications of isomaltulose syrup (dried) and the results of five batch analyses of isomaltulose syrup (dried) are presented in Table 1. These data demonstrate the consistency of the product.

According to FCC specifications (FCC, first published in its 7th edition), isomaltulose (syn.: Palatinose®) contains at least 98% (d.s.) 6-O-α-D-glucopyranosyl-D-fructofuranose. The remaining 2% are other saccharides, i.e., mainly trehalulose, fructose, glucose, and sucrose. The current FCC specifications of isomaltulose are shown in Annex 2. Evonik's corresponding specifications of isomaltulose syrup (dried) including a description of the method for quantitation of isomaltulose and the accompanying minor carbohydrate species are shown in Annex 3.

The subject of this GRAS notice is an isomaltulose syrup in dried form, with an isomaltulose content of not less than 80% (on a dry matter basis) (see Annex 3 for specifications of isomaltulose syrup (dried)). Among the carbohydrates which account for the remaining up to 20%, trehalulose is the quantitatively most important by-product (about 8.6–8.8%), followed by fructose (3.2–3.3%), sucrose that is the starting material

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[4] The current use of different types of alginates in food production and the regulatory status of alginates have been reviewed recently by the Organic Materials review Institute for the USDA National Organic Program (OMRI, 2015).

for the isomaltulose production (1.9–2.9%), glucose (1.7–1.9%), as well as isomelezitose and other oligosaccharides (1.2–1.3%).

(4) Data and information bearing on physical or other technical effect

Our GRAS notice does not include data and other information bearing on physical or other technical effect because such data and other information are not necessary to demonstrate safety.

Part 3 – Dietary exposure

(1) Intended Use of Isomaltulose Syrup

The range of intended food uses of isomaltulose syrup (dried) is as wide as the actual range of food uses of sucrose (liquid[5] and crystalline) that it may replace, be it partly or completely, depending upon the desired sensory and/or health-related properties.

Isomaltulose has similar food-technological properties as sucrose, except for its sweetness, which is about half that of sucrose (Südzucker, 2005), and its solubility in water, which also is about half that of sucrose at 50°C and even less at lower temperatures (Südzucker, 2005; i.e., GRN 000184 at pp. 9 and 10).

Isomaltulose syrup (dried) is expected to be used primarily for its physiological benefits. In certain applications, however, food-technological benefits also may lead to the use of isomaltulose syrup (dried).[6]

To best achieve the physiological benefits of isomaltulose, the total replacement of dietary sucrose is required. Hence, the theoretical maximum daily intake of isomaltulose corresponds to that of sucrose.

It has been noted that isomaltulose would replace only about 5–10% of sucrose because it is more expensive, less soluble in water, and less sweet than sucrose (Südzucker, 2005; i.e., GRN 000184 at p.12). Nevertheless, in this GRAS notice, the "worst-case" estimated daily intake of isomaltulose syrup is assumed, i.e., isomaltulose syrup (dried) substitutes for all sucrose that is consumed as a component of processed or ultra-processed foods and beverages, and by the consumer's direct addition to foods and beverages.

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5 Liquid sucrose has a dry substance content of about 66-68%. For specifications see, for example, https://www.cargillfoods.com/wcm/groups/public/@cseg/@food/@all/documents/document/na3034451.pdf.

6 An example may be fat-reduced chocolate-type products. The reduction of fat in chocolate is associated with a corresponding increase of the carbohydrate content. However, an increase of sucrose would lead to a chocolate which is too sweet. The use of isomaltulose syrup (dried), however, may allow for adjustment of the sweetness of such chocolate to the desired level.

(2) Actual daily intake of sucrose in the U.S.

Information about the intake of sucrose by the U.S. population may be derived either from data on its delivery for domestic food and beverage use or from population-based food consumption data derived from 24-hour recalls coupled with food composition data.

According to the latest USDA Sugar and Sweeteners Yearbook, the delivery of refined sugar (i.e., sucrose) for domestic food and beverage use was 68.4 pounds (dry basis) per person in 2014 (USDA, 2015). This corresponds to an average per capita sucrose disappearance of 85 g/d. The total losses of "added sugar and sweeteners" at retail and consumer level was 41% in 2010 (Buzby et al., 2014). Collectively, these data support a per capita sucrose intake of about 50 g/d or about 0.84 g/kg bw/d for a 60 kg consumer.

The daily intake of added sugars of any type was estimated at 82.9 g/person for the US population aged ≥ 4 years by the National Health and Nutrition Examination Survey (NHANES) 2003–2006 (Marriott et al., 2010; Marriott, 2011). Based on NHANES data from the 2007–2008 survey, the daily intake of added sugars among US residents ≥ 2 years of age was estimated at 76.7 g/d (95% CI: 71.6–81.9 g/d) (Welsh et al., 2011). From the NHANES survey of 2009/2010 which oversampled certain subgroups of the US population a slightly lower value of 73 g/d was reported (Martinez Steele et al., 2016). These figures represent total sugars, i.e., the sum of sucrose ("refined sugars"), high fructose corn syrup (HFCS), glucose syrup, dextrose, and, quantitatively negligible, honey and edible syrup. The USDA data on sweeteners deliveries indicate that in 2014 "refined sugar," i.e., sucrose, accounted for about 52% of the total amount of caloric sweeteners delivered for food and beverage use (USDA, 2015). Applying this value to the mean of the daily added sugars intake reported by Marriott et al. (2010) and Welsh et al. (2011), the average sucrose intake of the US population is estimated at 41.5 g/d or about 0.69 g/kg bw/d.[7]

Among U.S. adults, men consumed more energy (kilocalories) from added sugars than women, but the percentage of energy in the daily diet from added sugars was similar. With increasing age, the intake of energy from added sugars decreased (Ervin & Ogden, 2013).

Data of the two NHANES studies indicate a mean sucrose intake of 0.66 g/kg bw/d in the U.S. adult population. If it is assumed that the 90th percentile consumer ingests about twice as much of a common food as the mean consumer, the daily intake of added

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[7] From the NHANES 2009/2010 data, sucrose intakes of 38 g/d or about 0.63 g/kg bw/d are estimated.

sucrose by the so-called "heavy consumer" may be estimated at about 1.3 g/kg bw corresponding to about 78 g/day for a 60 kg consumer.

Direct data on the distribution of the intake of added sugars among 50–71 years old residents from eight States in the US are reported from the NIH-AARP health study (Tasevska et al., 2012). In the quintile with the highest intake of added sugars (of any kind), men and women consumed 30.6 and 31.9 g sucrose per 1000 kcal of total dietary energy intake. Applying an average energy intake of 32.6 kcal/kg bw/d (Swinburn et al., 2009), these added sucrose intakes correspond to 1.00 and 1.04 g/kg bw/d or 60 g and 62 g/day, respectively, for a 60 kg consumer.

Based on NHANES 2007–2008 data, the intake of added sugars (of any kind) among 2–5 years old children is 13.5% of their total energy intake (Welsh et al., 2011). Applying the average energy intake 326 kcal/kg bw also to this age group, this corresponds to an energy intake from added sugars intake of 4.4 kcal/kg bw, i.e., 1.1 g total added sugars per kg body weight. Evidently, sucrose would account for only part of this total added sugars intake. This means that also among 2–5 years old children which have a more homogenous pattern of food intakes than adults and hence a smaller intra-group variance of food intake, the ingestion of added sucrose is unlikely to exceed 1 g/kg bw/d.

(3) Estimated daily intake of isomaltulose syrup (dried)

It follows from the data presented in section (2) above that the intake of added sucrose, and hence the intake of isomaltulose as a sucrose substitute, is unlikely to exceed 1.0–1.3 g/kg bw/d, even among children and the so-called "heavy consumers."

Part 4 – Self-limiting levels of use

Isomaltulose syrup (dried) is intended to be used as a sucrose substitute in foods and beverages. Having a similar functionality, the levels of use of sucrose – although not specified – will typically act as the maximum use levels also for isomaltulose syrup (dried) or mixtures of isomaltulose syrup (dried) and sucrose.

Part 5 – Experience based on common use in food before 1958

Because the statutory basis for our conclusion of GRAS status is not through experience based on common use in food, our notice does not include evidence of a substantial history of consumption of the notified substance for food use by a significant number of consumers prior to January 1958.

Part 6 – Narrative

(1) Safety of isomaltulose and isomaltulose syrup

(a) Safety of *Protaminobacter rubrum*

The isomaltulose syrup that is the subject of this GRAS notice is obtained by the isomerization of sucrose which is catalyzed by non-viable, immobilized cells of *Protaminobacter rubrum* strain CBS 574.77.[8] This strain has been identified as the source of sucrose isomerase also in the application for approval of isomaltulose in the European Union (Cargill, 2003) and in the GRAS Notice of isomaltulose of 2005 (GRN 000184) (Südzucker, 2005).

This bacterial strain has later been identified as *Serratia plymuthica* (Goulter et al., 2012) and "*Protaminobacter rubrum*" is now considered a misnomer. In fact, the genus "*Protaminobacter*" has included only a few species, all of which were reclassified (Takeuchi et al., 1990). *Protaminobacter rubrum* CBS 574.77 has officially been renamed *Serratia plymuthica* CBS 574.77. Yet, as the classification of *Serratia plymuthica* as a species of *Serratia* has been questioned in the past (Ewing et al., 1959 as cited in Grimont & Grimont, 2006) and as "*Protaminobacter rubrum*" is still an accepted synonym of *Serratia plymuthica* (GBIF, 2016), this historically more widely used denomination is maintained throughout this present GRAS notice for the strain CBS 574.77 that is used by Evonik for the production of isomaltulose syrup.

Serratia plymuthica occurs widely in nature and has been found, for example, in the rhizosphere of *Brassica napus* and other plants. It is psychrophilic and it may even fail to grow at 37°C (Grimont & Grimont, 2006).

The safety of use of *Protaminobacter rubrum* as a source or recipient microorganism for genetic engineering has been evaluated by the German "Zentrale Kommission für Biologische Sicherheit" (German Commission for Biological Safety) in 2010 (BVL, 2010). This expert group rated *Protaminobacter rubrum* as belonging to risk group 1, i.e., the group of microorganisms that pose no risk for humans and the environment (BVL, 2010). In their report, the experts mention that, thus far, no diseases of humans, animals, or plants were described that could be associated with *Protaminobacter rubrum*

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[8] CBS, Centraalbureau voor Schimmelcultures.

and that the apathogenicity of this microorganism was demonstrated in an intravenous pathogenicity and toxigenicity study in rabbits and mice (Porter et al., 1991).

The safety of 21 plant-associated *Serratia plymuthica* strains (which however did not include strain CBS 574.77) was examined independently by the German Collection of Microorganisms and Cell Cultures (DSMZ). The DSMZ experts also stated that no pathogenicity factor is known and that there is no evidence that strains of *S. plymuthica* can cause infections in humans (Berg, 2006).

A conflicting publication cannot be ignored (Carrero et al., 1995). The authors report the isolation of *S. plymuthica* from six patients, five of which were considered to suffer from nosocomial infection. Yet, the antimicrobial susceptibility of *S. plymuthica* is well characterized (Stock et al., 2003) and all of the patients reported by Carrero and collaborators responded promptly to antibiotic therapy[9] (Carrerro et al., 1995).

In the present context, i.e., the safety assessment of isomaltulose syrup (dried), the possible infectivity of *S. plymuthica* has no direct relevance because the cells are inactivated by the immobilization procedure and because the heating steps in the downstream processing of isomaltulose syrup further ensure the absence of viable microorganisms in the end product.

The comprehensive search of the scientific literature failed to identify any reported toxigenic effects of *S. plymuthica*.

(b) Safety of isomaltulose

The safety data of isomaltulose were evaluated by experts when approval was sought for use of this sugar as a novel food in the European Union first by Cargill in 2003 and subsequently by Südzucker AG in 2004. The competent food assessment bodies of the UK and Germany, respectively, arrived at the conclusion that isomaltulose was safe as a novel food.[10] Since so-called "reasoned objections" were not received from the food assessment bodies of any other EU Member State, isomaltulose was subsequently authorized for food use without the need for an additional safety assessment by the European Food Safety Authority (EFSA) and without any restrictions of use (cf. Commission Decision 2005/457/EC addressed to Cargill and Commission Decision

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9 Cefotaxime; gentamycin/ampicillin.

10 For the summary of Cargill's application for the approval of isomaltulose, the first assessment of this application by the UK Advisory Committee and Novel Foods and Processes (ACNFP), and the second opinion of the Health Council of the Netherlands (VNN, 2004), see https://www.gezondheidsraad.nl/sites/default/files/Isomaltulose.pdf.

2005/581/EC addressed to Südzucker). Identical specifications apply for the isomaltulose products of Cargill and Südzucker AG.

Comprehensive descriptions of the safety data of isomaltulose were prepared by Cargill in support of its request for authorization of isomaltulose as a novel food in the European Union (Cargill, 2003) and by Südzucker in support of its GRAS Notice (Südzucker, 2005).

A summary of the then available safety data of isomaltulose has also been published by Food Standards Australia New Zealand as Attachment 2 in its final assessment report of isomaltulose as a novel food (FSANZ, 2007).[11]

The safety assessment of isomaltulose that formed the basis of GRAS Notice GRN 000184 and the authorizations of isomaltulose as a novel food in the European Union and Australia/NZ were based on published *in vivo* and *in vitro* studies demonstrating that ingested isomaltulose is completely hydrolyzed and absorbed in the small intestine as glucose and fructose (Macdonald & Daniel, 1983; Tsuji et al., 1986). Adverse effects were not reported in standard tests for mutagenicity (Baek et al., 1997), embryotoxicity/teratogenicity (Lina et al., 1997), and in acute and subchronic toxicity studies in rats (Yamaguchi et al., 1986, 1987; Lim et al., 1997; Jonker et al., 2002; Lina et al., 2002).

Since the authorizations of isomaltulose as novel food in the European Union and Australia/NZ, additional studies with isomaltulose in animals and humans were identified and retrieved as a result of a comprehensive search of the scientific literature published through April 8, 2016. These are summarized in this Part.

Since publication of the FDA's response letter to GRAS Notice GRN 000184 and since the authorizations of isomaltulose as novel food by the European Commission and the Food Standards Authority Australia/NZ, no additional safety studies of isomaltulose appeared in the scientific literature. However, there were further investigations mainly in rats, on the digestion, absorption, tolerance, and beneficial effects of isomaltulose.

A comparative study on the absorption and metabolism of 1-^{13}C labelled sucrose and isomaltulose showed that the absorption of isomaltulose is more protracted than that of

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[11] FSANZ noted that individuals with hereditary fructose intolerance (HFI) should completely avoid the intake of any fructose containing foods or foods that yield fructose upon digestion, such as sucrose and isomaltulose. However, since HFI sufferers are aware of their condition and are advised by their medical doctor or nutritionist about their dietary practices, no special labelling requirements were established for isomaltulose containing foods.

sucrose but that the total absorption (as reflected by the area under curve) is not significantly different between these two sugars (Tonouchi et al., 2011).

An inhibitory effect of isomaltulose on the *in vitro* hydrolysis of sucrose and soluble starch by the intestinal sucrose-isomaltulose complex and an inhibitory effect of isomaltulose on the absorption of glucose in the everted rat gut model were reported by a Japanese research group (Kashimura & Nagai, 2007a; Kashimura et al., 2008). Accordingly, the partial or complete substitution of sucrose in a semipurified diet with 14.95% starch and 40% sucrose reduced the weight gain of initially 38-week old mice by about 20–25% over an 8-week feeding period. The food intake did not differ between controls and treated groups but absolute values were not reported. Other treatment-related effects were not observed and none of the animals in any group exhibited diarrhea or loose stools (Kashimura & Nagai, 2007b). Assuming a food intake of 3 g/d (Yan et al., 2011), the isomaltulose intake was about 42 g/kg bw/d in the 40% isomaltulose group.

In a more recent study on effects on glucose metabolism of isomaltulose vs. sucrose feeding, adult male rats received semipurified diets with 60.5% starch (controls), and sucrose or isomaltulose for a period of 26 days (expt. 1) and 56 days (expt. 2). At a food intake of about 21.9 g/d (average of the two experiments) and a final body weight of 450 g, the rats consumed about 29.4 g/kg bw/d isomaltulose. Food intake was somewhat reduced for 2–4 days in rats switched to the isomaltulose diet. Adverse effects due to this treatment were not reported. In expt. 1, but not expt. 2, the mean body weight gain was significantly increased in sucrose-fed rats compared to both the starch- and isomaltulose-fed rats (Häberer et al., 2008, 2009).

(c) Safety of by-products in isomaltulose syrup

The predominant by-products in isomaltulose syrup (dried), which together account for less than 20% on dry matter basis are trehalulose, glucose, and fructose, and unreacted sucrose (Fig. 1). In addition, small amounts of isomelezitose and other oligosaccharides (1.2–1.3%), as well as traces of protein (< 1 ppm), have been reported (Table 1).

Among the sugar-type by-products, only trehalulose that occurs naturally in honey (Nakajima et al., 1990) deserves consideration because it is a structural isomer of sucrose, i.e., 1-O-α-D-glycopyranosyl-D-fructose. A liquid isomaltulose syrup in which trehalulose accounted for 45.7% of all sugars was administered orally at doses of 1.5, 3, and 4.5 g/kg bw/d to SD rats in a 26-week toxicity study (14 rats/sex/group) (Yamaguchi et al., 1987). There were no deaths attributed to treatment. In the males and females of the control, low- and mid-dose groups and in females of the high dose group, 14 rats/sex/group survived till the end of the study. Twelve males of the high-dose group survived for terminal investigations. Treatment did not affect the food intake and growth of the female animals. The food consumption of the high-dose males was significantly

reduced in the second half of the treatment period and body weights tended to be accordingly lower (the difference to controls was, however, not significant except in week 21). There were no reported adverse effects on standard hematological and clinicochemical blood parameters, organ weights, and gross and pathological findings (Yamaguchi et al., 1987).

Thus, the NOAEL of the tested isomaltulose syrup was 4.5 g/kg bw/d, i.e., the highest dose tested, corresponding to 3.2 g/kg bw/d isomaltulose syrup (dry matter). The NOAEL for the trehalulose present in this syrup was 1.47 g/kg bw/d.

Higher dietary levels of isomaltulose and trehalulose (additions of up to 56% at the expense of wheat starch) were applied in a caries study with male Sprague-Dawley rats which were 23 days old at start of the 73-day treatment period (10 rats per dose group). There were no significant differences reported in weight gains due to the isomaltulose and trehalulose treatments and there were no signs of adverse side-effects (Ooshima et al., 1991). The caries scores were low and did not differ between controls and the isomaltulose or trehalulose treatment groups.

Using the isolated everted intestinal segment model, trehalulose has been shown to be readily hydrolyzed and absorbed in the form of glucose and fructose, though not as rapidly as sucrose (Tsuji et al., 1986). This high absorption rate explains the high intestinal tolerance of trehalulose.

The oligosaccharides that account for about 1.2–1.3% of the isomaltulose syrup (dried) are not further characterized. Since sucrose is the only starting material of the production process, their presence does not raise safety concerns. The fact that the amount of free fructose in isomaltulose (dried) syrup exceeds that of the free glucose (~3.2% vs. ~1.8%) is compatible with the formation isomelezitose which has been found in isomaltulose syrup (0.5% of dry matter) (Véronèse & Perlot, 1999; Rose & Kunz, 2002). Such oligosaccharides occur naturally in different types of food and do not raise safety concerns because they either are hydrolyzed in the digestive tract[12] and absorbed or fermented by the gut microbiota in the distal parts of the GI tract.

The trace amounts of protein that have been detected in isomaltulose syrup (dried) might originate from the immobilized *Protaminobacter rubrum* biomass. Since this species is neither toxicogenic nor pathogenic (see Part 6(1)(a)), any such leakage lacks relevance for the safety of isomaltulose syrup (dried).

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12 Isomelezitose, for example, is very rapidly hydrolyzed by sucrose/isomaltulose (Hertel et al., 2000).

Any other by-products that would occur in isomaltulose syrup (dried) could originate only from the food-grade sucrose which is used as the raw material in Evonik's production process. Since under its intended conditions of use, isomaltulose syrup would substitute in the human diet for sucrose at an about 1:1 ratio, the daily intake of such by-products would remain essentially unchanged.

(d) Intestinal tolerance of isomaltulose in humans

Both ingested sucrose and isomaltulose are hydrolyzed in the small intestine by the same digestive enzyme, i.e., sucrose-isomaltulase, which is located on the brush border membrane. *In vitro,* this enzymatic hydrolysis proceeds at a lower speed for isomaltulose than for sucrose as a substrate. Although this difference may not be extrapolated directly to the *in vivo* situation, there is a possibility of an accumulation of non-digested isomaltulose in the GI-tract that could result in intestinal side-effects (bloating, loose stool, or even diarrhea), as reported to occur following the ingestion of excessive doses of lactose or polyols.

Summarized in Table 2 and Table 3 are the observations from studies with isomaltulose in adults and children, respectively. The results of these studies attest to the high intestinal tolerance of isomaltulose. Single isomaltulose doses of 41 g in children and 75 g in adults were tolerated without any side effects (Young & Benton, 2015; van Can et al., 2009). In several studies with adult volunteers, single doses of 50 g isomaltulose were given in aqueous solution after overnight fasting. It is known from studies on polyols that intestinal side-effects are more likely to occur under such conditions than when the same amount of test product is consumed together with a meal that would retard gastric emptying. Nonetheless, gastrointestinal disturbances were not reported except from 3 out of 13 participants of a recent study in which daily doses of 139 ± 13 g isomaltulose were consumed with a beverage (three portions per day) for seven consecutive days. At these high levels of intake (1.77 g/kg bw/d), only three out of the 13 subjects experienced mild GI symptoms during the first two days of treatment but there were no drop-outs because of such effects (Kahlhöfer et al., 2016).

Since ingested isomaltulose is rapidly enzymatically hydrolyzed and absorbed as glucose and fructose, several high doses could be consumed in the course of a day without risk of intestinal side-effects. Thus, daily doses of 1.0–1.3 g/kg bw/d are expected to be as well tolerated as corresponding doses of sucrose in adults and children.

(2) Safety of isomaltulose syrup (dried) under its intended conditions of use

As described in Part 3, paragraph 1, of this notice, isomaltulose syrup (dried) could substitute for sucrose in all food applications. The data presented in Part 3 demonstrate that the current total intake of added sucrose (not total sugars!) is unlikely to exceed 1.0–1.3 g/kg bw/d even among children and the so-called heavy consumers. A complete

substitution of sucrose by isomaltulose syrup (dried) would consequently result in an identical upper total daily intake of about 1.0–1.3 g. The data on intestinal tolerance, as presented in Part 3, demonstrate that adverse intestinal side effects are not to be expected under such conditions of use.

In conclusion, the publicly available information on the substantial equivalence of isomaltulose and sucrose with regard to bioavailability and nutritional value (as absorbed glucose and fructose), the absence of undesirable substances, the intended conditions of use, and human tolerance form a substantial basis for the general recognition of safety of the proposed use as a partial or complete substitute for sucrose in the daily diet of isomaltulose syrup (dried). An independent panel of scientific experts, qualified by training and experience to evaluate the safety of food ingredients, concluded that under the conditions of intended use in foods, Evonik's isomaltulose syrup (dried) is GRAS through scientific procedures. The panel's opinion is included at Annex 4.

Part 7 - List of supporting data and information

The references listed below are generally available.

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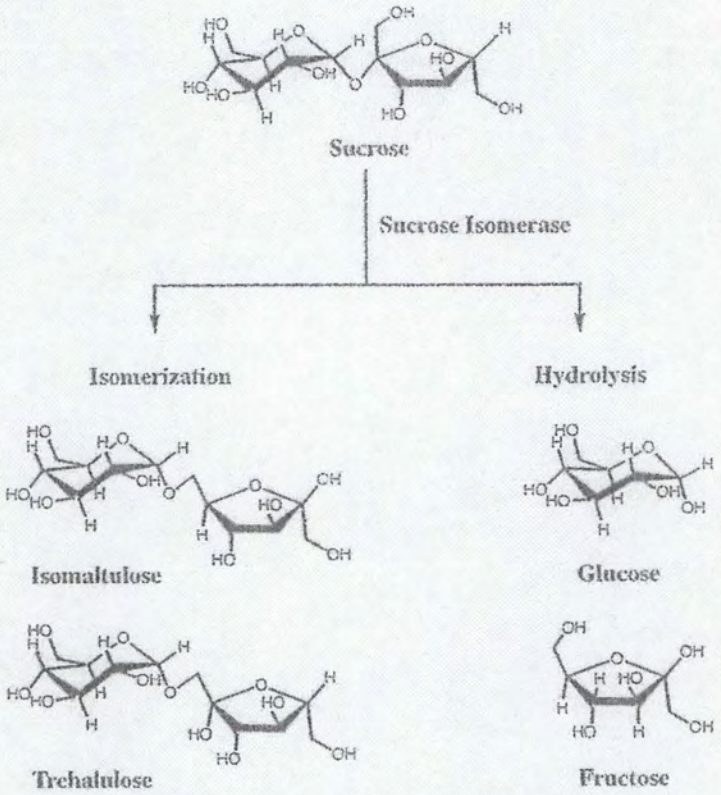


Fig. 1. Reaction Scheme for Sucrose Isomerase.

Table 1 **Specifications and typical composition of isomaltulose syrup (dried)**

	Specifications	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Isomaltulose	≥ 80%	82.7%	82.6%	82.4%	82.2%	82.1%
Trehalulose	≤ 12%	8.8%	8.7%	8.6%	8.7%	8.7%
Glucose	≤ 3%	1.9%	1.7%	1.8%	1.9%	1.8%
Fructose	≤ 4%	3.3%	3.2%	3.3%	3.2%	3.2%
Saccharose	≤ 4%	1.9%	2.5%	2.6%	2.8%	2.9%
Isomelezitose and Oligosaccharides	≤ 2%	1.3%	1.2%	1.2%	1.3%	1.3%
Protein[1]	≤ 1 mg/kg	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg
Water	≤ 7%	< 7%	< 7%	< 7%	< 7%	< 7%
Ash[2]	≤ 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%

Note. Except for water (on as is basis), all values are expressed on dry matter basis.

[1] For internal specification purpose only

[2] Residue on ignition (sulfated ash)

Table 2 **Tolerance of isomaltulose in adults**

Subjects	Study design	Treatments	Primary endpoints	Tolerance	References
Age, health condition	N, study design				
> 45 years healthy	n = 150 (n = 50/treatment) crossover	Breakfast with 40g glucose, sucrose or isomaltulose	Cognitive performance, mood	No adverse side effects noted	Young & Benton, 2014
23 ± 4 years healthy	n = 24 ♂ crossover (overnight fasted)	Milk-based drink (429 ml) With sucrose (50g) or with isomaltulose (50g) + sweeteners Water as control	Cognitive functions, glycemic response	No adverse side effects noted	Dye et al., 2010
53 ± 7 years healthy	♂ n = 10 ♀ n = 40 double blind, placebo controlled	40g/d sucrose or 20g/d sucrose + 20g/d isomaltulose for 12 weeks	LDL-Chol, HDL-Chol, TG, HOMA-IR, HbA_{1c}, BP, etc.	No adverse side effects noted	Okuno et al., 2010
Impaired glucose tolerance	♂ n = 14 ♀ n = 10 double, blind, crossover	17.5g/d isomaltulose for 12 weeks	Glucose tolerance blood lipids, blood pressure	No adverse side effects noted	Oizumi et al., 2007
Type-2 diabetics	n = 101 double blind, parallel design	50g/d sucrose or 50g/d isomaltulose for 12 weeks	HbA_{1c}, HOMA-IR, blood lipids, leptin, adiponectin	No adverse side effects noted	Brunner et al., 2012

Table 2 **Tolerance of isomaltulose in adults (continued)**

Type-1 diabetics	n = 7	0.6g/kg bw single dose (~41.8 ± 1.3g/subject)	Blood glucose, lactate, pH and cardiorespiratory parameters before and after exercise	No adverse side effects noted	Bracken et al., 2012
31 ± 4 years healthy	n = 10 single blind, crossover	1 x 75g sucrose 1 x 75g iso-maltulose Single doses with a drink following overnight fasting	Glycemic and insulinemic response	No adverse side effects noted	Van Can et al., 2009
23 ± 0.4 years healthy	n = 8 Random sequence	1 x 50g sucrose 1 x 50 g iso-maltulose Single dose with 150ml water (consumed in 2-3 min.)	Glycemic and insulinemic response	No adverse side effects noted	Kawai et al., 1985
18-35 years	n =18 5 treatments with crossover design	50g sucrose 50g isomaltulose Green Tea Green Tea with 50g sucrose Green Tea with 50g isomaltulose	Glycemic and insulinemic response	No adverse side effects noted	Suraphad et al., 2015b
20-35 years BMI 25.0-29.9	n = 12 single blind, crossover	40g sucrose 40g isomaltulose	Glycemic response	No adverse side effects noted	Suraphad et al., 2015a

Table 2 **Tolerance of isomaltulose in adults (continued)**

Type-1 diabetics 35 ± 2 years	n = 8	75g glucose or 75g isomaltulose with 750ml water	Blood glucose, lactate, hormones, fatty acids etc.	No adverse effects noted	West et al., 2011
Healthy males 31.6 ± 0.5 years	n = 7 two randomized crossover expts.	Liquid formula (500ml) with dextrin (controls) or 35 g ismaltulose and 27.9g dextrin (test)	Plasma glucose, insulin, free fatty acids, carbohydrate and fat oxidation rates	No adverse effects noted	Arai et al., 2007
Healthy males 27 ± 2 years	n = 10 crossover with randomly assigned sequence of treatments	Aqueous 8.5% solution of sucrose (51g/600ml) or 8.5% solution of isomaltulose (51g/600ml), consumed within 5 minutes	Plasma glucose, insulin, free fatty acids, carbohydrate and fat oxidation rates	No adverse effects noted	Achten et al., 2007
19 - 26 years	n = 13 ♂ crossover single blind, randomized	139 g/d maltodextrin + sucrose 139 g/d isomaltulose ingested with beverage in three portions/day	Insulin sensitivity, Insulin secretion	GI-disturbance during first 2 days on isomaltulose treatment in 3 subjects	Kahlhöfer et al., 2016

Table 3 Tolerance of isomaltulose in children

Age, health condition	Study design	Treatments	Primary endpoints	Tolerance	References
5-11 years healthy	n = 75, crossover, double-blind	41g glucose with breakfast 41g isomaltulose with breakfast	Cognitive functions	No adverse effects noted	Young & Benton, 2015
5 - 6 years healthy	n = 100 partly randomized, crossover	"growing-up milk" with 5g isomaltulose/serving (2 servings/day)	Cognitive functions	No adverse effects noted	Sekartini et al., 2013
5 - 6 years	n = 30 double blind, crossover (4 periods)	"growing-up milk" containing/lactose, sucrose and glucose + isomaltulose (4.48g) or sucrose/glucose	Cognitive functions	No adverse effects noted	Mohd Taib et al., 2012

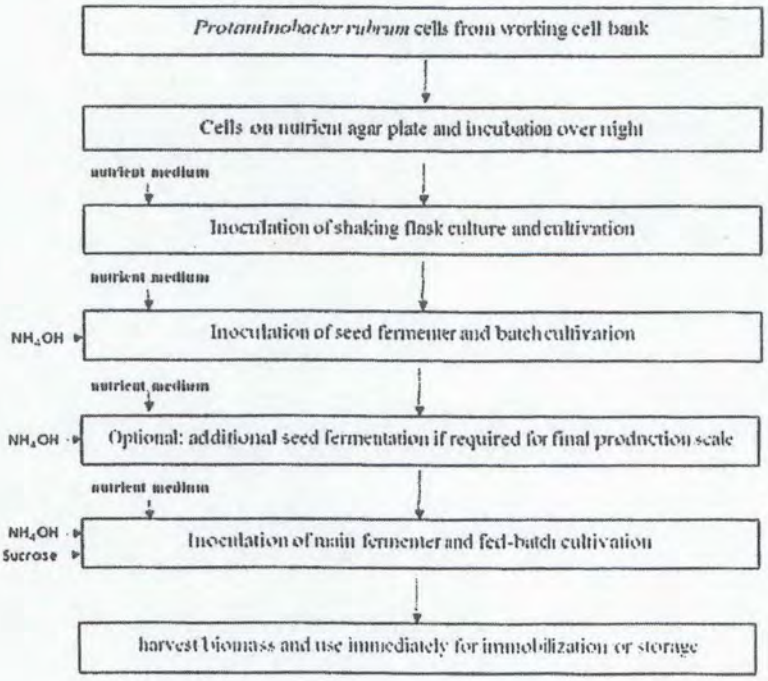
Annex 1

Production process of isomaltulose syrup (dried)

1. Preparation of the biocatalyst

The preparation of the biocatalyst is carried out in three steps (Figure 1). Starting from a Working Cell Bank vial, the cells of *Protaminobacter rubrum* CBS574.77 are incubated over night on a nutrient agar plate. The cells are then transferred in a 1-L shake flask (seed phase 1). After reaching the crossover criteria ((≥14h; <24h) and OD_{600} >10), the seed phase 1 is transferred in a 10-L stirred tank fermenter (seed phase 2). The final third step is carried out in a 500-L stirred tank fermenter, with sucrose feeding at a rate of 20 g/L/h. After 15-20 h the termination criterion of the fermentation process (OD_{600} > 90) is reached. The process is stopped and the *Protaminobacter rubrum* biomass is harvested for the subsequent immobilization process.

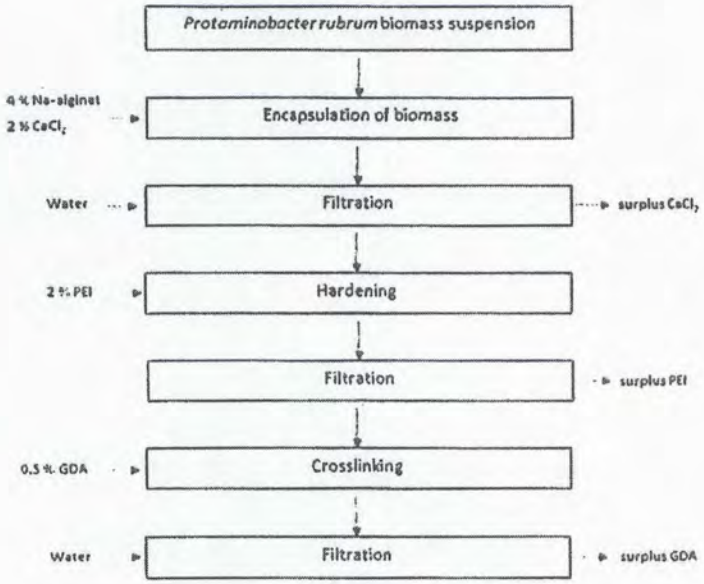
Figure 1: Preparation of the biocatalyst



2. Immobilization of the biocatalyst

The immobilization process comprises two steps, i.e. immobilization of the biomass in calcium alginate and hardening of the calcium alginate beadlets (Figure 2). For the first step sodium alginate is dissolved in hot (> 80°C) deionized water. After cooling the alginate solution to room temperature, the suspension of the biomass is mixed with the alginate-solution in a 50:50 ratio and stirred for at least 15 minutes. This mixture is then passed through a particle formation device and dripped into a stirred aqueous solution of calcium chloride (2% w/w). A stirrer with low shear force is applied to avoid disruption of the formed particles. The hardening process consists of two steps. In the first step, the obtained calcium alginate/biomass spheres are cured with 2% (w/w) polyethlyenimine at a pH of 5.5 under constant gentle stirring for 10 minutes. The beads are then recovered on a sieve (0.5 mm) and are washed with water. In the following second step, the particles are treated with an 0.5% (w/w) glutaraldehyde solution. Thereafter, the biocatalyst beads are washed with water and may be stored in water at 4°C for up to several weeks before use.

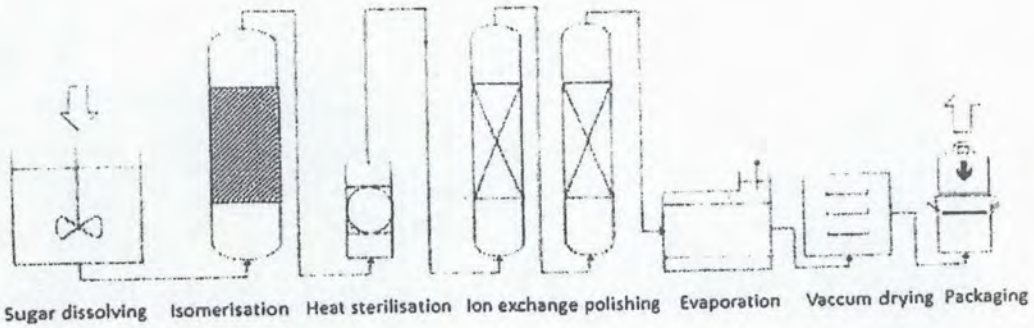
Figure 2: Immobilization of the biocatalyst



3. Production of the isomaltulose syrup (dried)

The immobilized biocatalyst is used for the production of the isomaltulose syrup (Figure 3). In a first step, an aqueous 35-45% sucrose solution is passed through a temperature-controlled column reactor (heated to 20-35°C) which is charged with the immobilized *Protaminobacter rubrum* bio-catalyst. The isomerization reaction produces isomaltulose with a small amount of other mono- and disaccharide by-products (trehalulose, glucose, fructose, isomelezitose) as well as trace amounts of trisaccharides. The eluate of the column reactor is heat-sterilized and deionized by passing it through a cation and an anion exchange column. The eluate is then concentrated by evaporation and vacuum dried. The obtained isomaltulose syrup (dried) is packed and stored under standard conditions.

Figure 3: Production of the Isomaltulose syrup



Annex 2

FCC IX Specifications of isomaltulose

C_S = concentration of 1,6-GPS or 1,1-GPM in the *Standard solution* (mg/mL) [Calculated based on the declared 1,6-GPS or 1,1-GPM content of USP Isomalt RS]

C_U = concentration of sample in the *Sample solution* (mg/mL)

Calculate the percentage of total hydrogenated mono- and disaccharides (%THS) in the sample taken using the following equation:

$$\%THS = A + B$$

A = sum of the percentages of 1,6-GPS and 1,1-GPM in the sample taken calculated above

B = sum of the percentages of mannitol and sorbitol in the sample, determined separately in the *Mannitol and Sorbitol* test procedure below

Acceptance criteria: NLT 98.0% of total hydrogenated mono- and disaccharides (%THS) and NLT 86% of the mixture of 1,6-GPS and 1,1-GPM, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **LEAD,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB
  Acceptance criteria: NMT 1 mg/kg
- **NICKEL,** *Nickel Limit Test, Method II,* Appendix IIIB
  Acceptance criteria: NMT 2 mg/kg

Organic Impurities

- **MANNITOL AND SORBITOL**
  Mobile phase: Degassed water
  Standard solution: 0.1 mg/mL each of USP Sorbitol RS and USP Mannitol RS
  System suitability solution: 20 mg/mL, 0.1 mg/mL, and 0.1 mg/mL of USP Isomalt RS, USP Sorbitol RS, and USP Mannitol RS, respectively
  Sample solution: 20 mg/mL
  Chromatographic system, Appendix IIA
  Mode: High-performance liquid chromatography
  Detector: Refractive index
  Column: 7.8-mm × 30-cm analytical column[1] and a 4.6-mm × 3-cm guard column, both packed with a strong cation-exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the calcium form, about 9 µm in diameter
  Column temperature: 80 ± 1°
  Flow rate: About 0.5 mL/min
  Injection volume: About 20 µL
  System suitability
  Sample: *System suitability solution*
  Resolution: NLT 2.0 between 1,1-GPM and 1,6-GPS
  Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the for 1,6-GPS and 1,1-GPM. [NOTE—The typical retention time of 1,1-GPM is about 12.3 min; the relative retention times are about 1.2 for 1,6-GPS, about 1.6 for mannitol, about 2.0 for sorbitol, and 1.0 for 1,1-GPM.]

  Separately calculate the percentages of mannitol and sorbitol in the sample taken by the following formula:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100\%$$

r_U = peak response for mannitol or sorbitol from the *Sample solution*

r_S = peak response for mannitol or sorbitol from the *Standard solution*

C_S = concentration of mannitol or sorbitol in the *Standard solution* (mg/mL)

C_U = concentration of sample in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3% mannitol and NMT 6% sorbitol

- **REDUCING SUGARS**
  Alkaline tartrate solution: Dissolve 34.6 g of potassium sodium tartrate (Rochelle salt) and 10 g of sodium hydroxide in water, dilute to 100 mL, let stand 2 days, and filter through glass wool.
  Sample: 7 g
  Analysis: Dissolve the *Sample* in 35 mL of water in a 400-mL beaker, and mix. Add 25 mL of cupric sulfate TS and 25 mL of *Alkaline tartrate solution.* Cover the beaker with glass and heat the mixture at such a rate that it comes to a boil in approximately 4 min and boils for exactly 2 min. Filter the precipitated cuprous oxide through a tared Gooch crucible previously washed with hot water, ethanol, and ether, and dried at 100° for 30 min. Thoroughly wash the collected cuprous oxide on the filter with hot water, then with 10 mL of ethanol, and finally with 10 mL of ether, and dry at 100° for 30 min. Weigh the filter containing the cuprous oxide.
  Acceptance criteria: The weight of the cuprous oxide does not exceed 50 mg (NMT 0.3% (as glucose)).

SPECIFIC TESTS

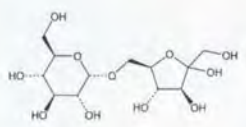
- **WATER,** *Water Determination,* Appendix IIB
  Acceptance criteria: NMT 7.0%
- **RESIDUE ON IGNITION (SULFATED ASH),** Appendix IIC
  Sample: 5 g
  Acceptance criteria: NMT 0.05%

Isomaltulose

First Published: FCC 7

Palatinose

6-*O*-α-D-Glucopyranosyl-D-fructofuranose, Monohydrate



$C_{12}H_{22}O_{11} \cdot H_2O$ Formula wt 360.6

CAS: [13718-94-0]

UNII: 43360LXH8N [isomaltulose monohydrate]

DESCRIPTION

Isomaltulose occurs as a white or colorless, crystalline, sweet substance with a faint, characteristic odor. It is a reducing disaccharide consisting of one glucose and one fructose moiety linked by an α-1,6-glycosidic bond. Isomaltulose is soluble in water.

Function: Nutritive sweetener; formulation and texturizing aid

Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

• **A. PROCEDURE**

Acceptance criteria: The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the *Assay*.

• **B. THIN-LAYER CHROMATOGRAPHY**, Appendix IIA

Solution A: 1 g/L of sodium periodate

Solution B: Absolute ethanol, sulfuric acid, acetic acid, and anisaldehyde (90:5:1:1)

Standard solution: 5 mg/mL of USP Isomaltulose RS

Sample solution: 5 mg/mL

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator having optimal intensity at 254 nm

Developing solvent system: Ethyl acetate, pyridine, acetic acid, propionic acid, and water (10:10:1:1:2)

Application volume: 1 µL

Analysis: Separately apply the *Sample solution* and the *Standard solution* to the chromatographic plate and thoroughly dry the starting points in warm air. Develop over 10 cm using the *Developing solvent system*, dry the plate in a current of hot air, and dip for 3 s in *Solution A*. Dry the plate in hot air. Dip the plate for 3 s in *Solution B*. Dry the plate in a current of hot air until colored spots become visible. The background color may be brightened by exposure to warm steam. Examine in daylight.

Acceptance criteria: The chromatograms obtained from the *Standard solution* and the *Sample solution* show principal spot(s) similar in position and color.

ASSAY

• **PROCEDURE**

Mobile phase: 70% acetonitrile in water, degassed. [NOTE—The exact concentration can be 60%–75%, depending on the kind and condition of column used.]

Standard solution: 100 mg/mL of USP Isomaltulose RS

Sample solution: 100 mg/mL

System suitability solution: 76 mg/mL of USP Isomaltulose RS; 4 mg/mL each of trehalulose and isomaltose; 5 mg/mL each of USP Fructose RS, USP Sucrose RS, and USP Dextrose RS

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index

Column: 4.6-mm × 25-cm amino phase analytical column and 4.6-mm × 1-cm amino phase precolumn (ZORBAX-NH_2, or equivalent, analytical column and precolumn)

Column temperature: Ambient

Flow rate: 1.0–1.8 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirement 1: The resolution between isomaltulose and the previous peak (sucrose) and the following peak (trehalulose) is NLT 1.4.

Suitability requirement 2: The relative standard deviation of the isomaltulose peak response is NMT 2.0%.

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for isomaltulose.

Calculate the percentage of Isomaltulose in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for isomaltulose from the chromatogram of the *Sample solution*

r_S = peak response for isomaltulose from the chromatogram of the *Standard solution*

C_S = concentration of isomaltulose in the *Standard solution* (mg/mL)

C_U = concentration of Isomaltulose in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 98.0% of isomaltulose, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

• **LEAD**, *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I*, Appendix IIIB

Acceptance criteria: NMT 0.1 mg/kg

Organic Impurities

• **OTHER SACCHARIDES**

Mobile phase: Prepare as directed in the *Assay*.

Standard solution: 98.5 mg/mL of USP Isomaltulose RS; 0.6 mg/mL each of trehalulose and isomaltose; 0.1 mg/mL each of USP Fructose RS, USP Sucrose RS, and USP Dextrose RS

Sample solution: 100 mg/mL

System suitability solution: Prepare as directed in the *Assay*.

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index

Column: 4.6-mm × 25-cm amino phase analytical column and 4.6-mm × 1-cm amino phase precolumn (ZORBAX-NH_2, or equivalent, analytical column and precolumn)

Column temperature: Ambient

Flow rate: 1.0–1.8 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirement: The resolution between isomaltulose and the previous peak (sucrose) and the following peak (trehalulose) is NLT 1.4.

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the

chromatograms, and measure the responses for the saccharides. [NOTE—The approximate retention time of isomaltulose is 7.7 min; the approximate relative retention times are 0.67 for fructose, 0.74 for dextrose, 0.92 for sucrose, 1.00 for isomaltulose, 1.10 for trehalulose, and 1.2 for isomaltose.]

Calculate the percentage of each saccharide in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for the saccharide from the chromatogram of the *Sample solution*

r_S = peak response for the saccharide from the chromatogram of the *Standard solution*

C_S = concentration of the saccharide in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2% of other saccharides (total), calculated on the anhydrous basis

SPECIFIC TESTS

- **CONDUCTIVITY ASH**

[NOTE—For preparation of all solutions, twice-distilled or deionized water with a conductivity of less than 2 µS/cm should be used.]

Sample: 31.3 g, based on the dry substance

Analysis: Dissolve the *Sample* in carbon dioxide-free water and dilute to 100 mL. Using an appropriate conductivity meter that has been standardized with a potassium chloride conductivity calibration standard, measure the conductivity of the solution while gently stirring with a magnetic stirrer.

The conductivity ash, in g/100 g of solution, is calculated by the formula:

$$\text{Result} = F \times [C_1 - (0.35 \times C_{WATER})]$$

F = factor to convert to Conductivity Ash % (0.0006)

C_1 = measured conductivity of the solution containing the *Sample* at 20° (µS/cm)

C_{WATER} = specific conductivity of the water at 20° (µS/cm)

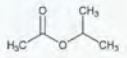
Acceptance criteria: NMT 0.01 g/100 g

- **WATER**, *Water Determination, Method 1a*, Appendix IIB

Acceptance criteria: NMT 6.0%

Isopropyl Acetate

First Published: Prior to FCC 6
Last Revision: FCC 7



$C_5H_{10}O_2$ Formula wt 102.13
FEMA: 2926
UNII: 1Y67AFK870 [isopropyl acetate]

DESCRIPTION

Isopropyl Acetate occurs as a colorless, mobile liquid.
Odor: Ethereal
Solubility: Miscible in alcohol, ether, most fixed oils; 1 g dissolves in 72 mL water.
Boiling Point: –88°
Function: Flavoring agent

IDENTIFICATION

- **INFRARED ABSORPTION**, *Spectrophotometric Identification Tests*, Appendix IIIC

Reference standard: USP Isopropyl Acetate RS
Sample and standard preparation: *F*
Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.

ASSAY

- **PROCEDURE:** Proceed as directed under *M-1b*, Appendix XI.

Acceptance criteria: NLT 99.0% of $C_5H_{10}O_2$

SPECIFIC TESTS

- **ACID VALUE, FLAVOR CHEMICALS (OTHER THAN ESSENTIAL OILS)**, *M-15*, Appendix XI

Acceptance criteria: NMT 2.0

- **SPECIFIC GRAVITY:** Determine at 25° by any reliable method (see *General Provisions*).

Acceptance criteria: Between 0.866 and 0.869

Isopropyl Alcohol

First Published: Prior to FCC 6
Last Revision: Third Supplement, FCC 7

2-Propanol
Isopropanol

C_3H_8O Formula wt 60.10
CAS: [67-63-0]

UNII: ND2M416302 [isopropyl alcohol]

Annex 3

Specifications of isomaltulose syrup (dried)

Isomaltulose syrup (dried)

Description

Isomaltulose syrup (dried) occurs as a white or colorless, crystalline, sweet product. Isomaltulose is a reducing disaccharide consisting of one glucose and one fructose moiety linked by an α-1,6-glycosidic bond. Isomaltulose is soluble in water.

Function: Nutritive sweetener; formulation and texturizing aid

Packaging and Storage: Store in well-closed containers.

Assay

- **PROCEDURE**

Mobile phase: Mix 452g acetonitrile with 205g acetone and 160 mL water, degassed.

Standard solution: 100 mg/mL of USP Isomatlulose RS

Sample solution: 100 mg/mL

System suitability solution: 76 mg/mL of USP

Isomatlulose RS; 4 mg/mL each of trehalulose and isomaltose; 5 mg/mL each of USP Fructose RS, USP Sucrose RS and USP Dextrose RS

Chromatographic system:

Mode: High-performance liquid chromatography

Detector: Refractive index (35°C)

Column: 4.6-mm x 25-cm amino phase analytical column (Waters Spherisorb, 5 µm)

Column temperature: 30°C

Flow rate: 2.0 mL/min

Injection size: 10 µL

System suitability

Sample: System suitability solution

Suitability requirement 1: The resolution between isomaltulose and the previous peak (sucrose) and the following peak (trehalulose) is NLT 1.14

Suitability requirement 2: The relative standard deviation of the isomaltulose peak response is NMT 2.0

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for isomaltulose.

Calculate the percentage of Isomaltulose in the sample taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response for isomaltulose from the chromatogram of the *Sample solution*

r_s = peak response for isomaltulose from the chromatogram of the *Standard solution*

C_s = concentration of isomaltulose in the *Standard solution* (mg/mL)

C_u = concentration of isomaltulose in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 80% of isomaltulose, calculated on the anhydrous basis

Impurities

Inorganic Impurities

- **LEAD**, *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

Acceptance criteria: NMT 0.1 mg/kg

Organic Impurities

- **OTHER SACCHARIDES**
  Mobile phase: Prepare as directed in the Assay.
  Standard solution: 98.5 mg/mL od USP Isomaltulose RS; 0.6 mg/mL each of trehalulose and isomaltose 0.1 mg/mL each of USP Fructose RS, USP Sucrose RS, and USP Dextrose RS

Sample Solution: 100 mg/mL

System suitability solution: Prepare as directed in the *Assay*.

Chromatic System: Proceed as directed in *Assay*.

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the saccharides. [NOTE - The approximate retention time of isomaltulose is 12.5 min; the approximate relative retention times are 5.2 min. for fructose, 6.3 min. for dextrose, 10.5 min. for sucrose, and 14.3 min. for trehalulose.]

Calculate the percentage of each saccharide in the sample taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response for the saccharide from the chromatogram of the *Sample solution*

r_s = peak response for the saccharide from the chromatogram of the *Standard solution*

C_s = concentration of the saccharide in the *Standard solution* (mg/mL)

C_u = concentration of the saccharide in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 20% of other saccharides (total), calculated on the anhydrous basis

Annex 4

EXPERT PANEL OPINION

The Generally Recognized as Safe (GRAS) Status of the Use of Isomaltulose Syrup (Dried) as a Sucrose Substitute in Foods and Beverages

A. INTRODUCTION

The undersigned, an independent panel of experts qualified by their scientific training and national and international experience to evaluate the safety of food and food ingredients (the "Expert Panel"), was specially convened by Evonik Creavis GmbH ("Evonik") to evaluate the safety and Generally Recognized as Safe (GRAS) status of the use of isomaltulose syrup (dried) as a sucrose substitute in foods and beverages (substitution at a ratio of up to 1:1, w/w). For purposes of the Expert Panel's evaluation, safe or safety means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i).

Albert Bär PhD of Bioresco Ltd. performed a comprehensive search of the scientific literature through April 1, 2016, relating to the safety of isomaltulose syrup (dried). Dr. Bär summarized the results of the literature search and prepared a safety dossier, "Dossier supporting the safety and Generally Recognized as Safe (GRAS) status of isomaltulose syrup (dried) for use as a sucrose substitute in foods and beverages" (June 15, 2016) for consideration by the Expert Panel.

The Expert Panel (Drs. Borzelleca, Nicolosi, and Pariza) individually and collectively critically evaluated the safety dossier and other available data and information that the members of the Expert Panel believed to be pertinent to the safety of the intended use of isomaltulose syrup (dried) as a sucrose substitute. The safety dossier included a summary of information and data, both favorable and unfavorable, compiled from the search of the scientific literature, information on the method of manufacture, product specifications, stability and analytical data, intake estimates for isomaltulose syrup (dried) under its intended conditions of use, and a safety assessment including the results of an earlier safety assessment of isomaltulose by a GRAS expert panel (Südzucker, 2005) and authoritative food safety assessment bodies (ACNFP, 2004; FSANZ, 2007).

Following its critical evaluation of this data and information, the Expert Panel unanimously concluded that Evonik's isomaltulose syrup (dried), produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is safe for use as a sucrose substitute in foods and beverages (substitution at a ratio of up to 1:1, w/w). The Expert Panel further concluded unanimously that the use of Evonik's isomaltulose syrup (dried) as a sucrose substitute in foods and beverages is GRAS based on scientific procedures. It is also the unanimous opinion of this Expert Panel that other qualified experts would concur with these conclusions.

Summarized below are the data, information, and interpretive analysis supporting the Expert Panel's conclusions.

B. SUMMARY AND BASIS FOR THIS GRAS DETERMINATION

In the US, isomaltulose produced by isomerization of sucrose with an immobilized enzyme preparation consisting of non-viable cells of *Protaminobacter rubrum* (strain (CBS 574.77) and having a purity of ≥ 98% is considered GRAS for use as a sucrose substitute. SÜDZUCKER intends isomaltulose to be used as a nutritive sweetener that would totally or partially replace sucrose or other highly digestible carbohydrates in certain foods and at use levels as specified in GRAS Notice GRN 000184. The notifier estimated the intake of isomaltulose from its combined proposed uses at about 3 – 6 g/person/day (FDA, 2006).

(1) In the European Union, isomaltulose with a purity of ≥ 98% and produced by an enzyme preparation from *Protaminobacter rubrum* is authorized as a novel food (EU Commission, 2005a,b) based on the safety assessment by the UK Advisory Committee on Novel Foods and Processes (ACNFP, 2004). Similarly, Food Standards Australia New Zealand authorized isomaltulose on the basis of its own safety assessment and intake estimates as a novel food without restrictions of use (FSANZ, 2007).

(2) Since acceptance of isomaltulose as a safe food ingredient in the US, EU and Australia/New Zealand on the basis of published standard toxicity tests, no additional safety studies on isomaltulose were published and there were no other published studies that would question the safety or the tolerance in humans of isomaltulose.

(3) Numerous human studies have been published on potential beneficial effects of isomaltulose owing to its slower, yet essentially complete digestion to readily absorbed glucose and fructose, and thus its reduced glycemic effect. Intestinal side effects were not reported from studies in which single doses of 75 g isomaltulose were ingested with water and with or without a meal after overnight fasting (van Can et al., 2012; West et al., 2011). However, three out of 13 healthy men consuming a total of 139 ± 13 g isomaltulose with a beverage consumed in three portions between meals experienced transient gastrointestinal disturbances during the first two days of a 7-day study (Kahlhöfer et al., 2016). Children 5 – 11 years of age tolerated the ingestion of 41 g isomaltulose (single dose) without intestinal side-effects (Young & Benton, 2015).

Data of the NHANES 2003 – 2006 and 2007 – 2008 surveys, the NIH-AARP health study and associated information indicate that the total daily intake of sucrose is unlikely to exceed about 1.3 g/kg bw/d (91 or 78 g/day for a 70 kg or 60 kg subject, respectively) in the so-called heavy consumer (children and adults alike) (Marriott et al., 2010, 2011; Welsh et al., 2011; Martinez Steele et al., 2016). Even in the unlikely case of a consumer who would substitute his entire

daily sucrose intake by isomaltulose syrup (dried) at a 1:1 (w/w) ratio, intestinal side-effects are therefore unlikely to occur.

(4) *Protaminobacter rubrum* (syn. *Serratia plymuthica*) is accepted as safe (Berg, 2006, BVL, 2010); thus, its use in the production of ismolatulose syrup (dried) is without risk. Note that only heat-inactivated and immobilized cells of *Protaminobater rubrum* are used in the production of isomaltulose syrup (dried).

(5) Isomaltulose syrup (dried) has a minimum isomaltulose content of not less than 80% calculated on the anhydrous basis. The by-products that account for the remaining up to 20% are mostly trehalulose, glucose, fructose and sucrose. Trehalulose (1-O-α-D-glucopyranosyl-D-fructose), like isomaltulose, is hydrolyzed in the small intestine to glucose and fructose. Trehalulose occurs naturally in certain foods. Thus, it does not raise safety concerns (see also Yamaguchi et al., 1987; Tsuji et al., 1986). Oligosaccharides consisting of glucose and fructose only (since sucrose is the only starting material of the process) occur in isomaltulose syrup (dried) at a concentration of about 1.2 – 1.3%. Such oligosaccharides occur naturally in different types of food and do not raise safety concerns because they either are hydrolyzed and absorbed in the small intestine or are fermented by the gut microbiota in the distal parts of the GI tract.

C. CONCLUSION

We, the undersigned Expert Panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the use as a sucrose substitute in foods and beverages (substitution at a ratio of up to 1:1, w/w) of Evonik's isomaltulose syrup (dried), produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, and unanimously conclude that such use is safe.

We further unanimously conclude that the use as a sucrose substitute in foods and beverages (substitution at a ratio of up to 1:1, w/w) of Evonik's isomaltulose syrup (dried), produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is Generally Recognized As Safe (GRAS) based on scientific procedures.

It is our unanimous opinion that other qualified experts would concur with these conclusions.

By (b) (6)

29 September 2016

Joseph F. Borzelleca, Ph.D. Date
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Virginia Commonwealth University School of Medicine
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By:

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By:

Michael W. Pariza, Ph.D. Date
Professor Emeritus, Department of Food Science
Director Emeritus, Food Research Institute
University of Wisconsin-Madison

C. CONCLUSION

We, the undersigned Expert Panel members, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the use as a sucrose substitute in foods and beverages (substitution at a ratio of up to 1:1, w/w) of Evonik's isomaltulose syrup (dried), produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, and unanimously conclude that such use is safe.

We further unanimously conclude that the use as a sucrose substitute in foods and beverages (substitution at a ratio of up to 1:1, w/w) of Evonik's isomaltulose syrup (dried), produced consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is Generally Recognized As Safe (GRAS) based on scientific procedures.

It is our unanimous opinion that other qualified experts would concur with these conclusions.

By: _____ _____

Joseph F. Borzelleca, Ph.D. Date
Professor Emeritus Pharmacology & Toxicology
Virginia Commonwealth University School of Medicine
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By: (b) (6) _____ 29 September 2016

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Michael W. Pariza, Ph.D. Date
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D. REFERENCES

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SUBMISSION END