PALATINIT

Member of the Group SÜDZUCKER

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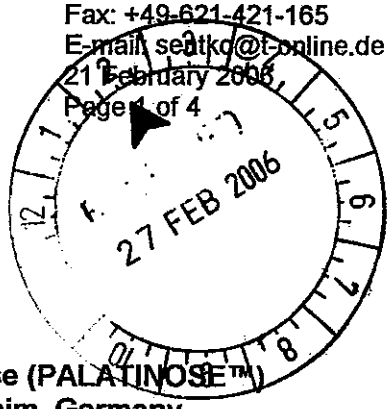
21 February 2006



Food Standards Australia New Zealand
Att.: Standards Management Officier
PO Box 7186
Canberra BC ACT 2610
Tel +61 2 6271 2222

NOVEL FOOD APPLICATION: Isomaltulose (PALATINOSE™)
Submitted by: PALATINIT GmbH, Mannheim, Germany
Member of the Südzucker Group

Dear Standards Management Officer,

Please find attached PALATINIT's/SÜDZUCKER's application for the approval of isomaltulose (PALATINOSE™) as a novel food in Australia/New Zealand.
As requested, the application is set out in the required format. The check list is attached to this letter (attachment 1). In attachment 2 you will find a document describing the structure of the submission. The application including copies of most of the references cited consists of 3 volumes. You will find 3 hard copies and one electronic copy (CD). Some annexes and references are confidential. They are clearly marked as such and collated in a separate section/file titled "confidential information". We are happy to share the included confidential information with Food Standards Australia New Zealand for their review. However, this information may not be placed in the public domain. It is commercial confidential information either in the form of primary information that is proprietary to PALATINIT / SÜDZUCKER that has a commercial value that would be lost if the information were disclosed to our competitor or in the form of non-public third party evaluations from other regulatory Authorities that assessed this information. We sincerely hope that this will be accepted by FSANZ.

We understand that the preferred time for payment of the initial assessment fee ($4000) is when the application is lodged. We assume that in doing so, we will receive an invoice.

For the best possible communication between FSANZ and PALATINIT, we advise that Dr. Simon Brooke-Taylor, PO Box 544, Bright Vic 3741, Ph/Fax 03 5750 1893, Mobile 0411 156 773, Email: simon@brooketaylor.com.au should be FSANZ's primary point of contact in case of questions about the application.

Best regards

Anke Sentko
Head of Regulatory Affairs

Hans-Ulrich Frech
General Manager

Encl.: Attachments 1 and 2 and enclose of 3 hard copy files and a CD

ATTACHMENT 1:

Application to develop or vary the *Australia New Zealand Food Standards Code*

Novel Foods Checklist

PART 1 General information	Data provided	Data Not provided	Omission explained
1.1 Applicant	✓		
(a) Name	✓		
(b) Address	✓		
(c) Contact	✓		
(d) Business	✓		
1.2 Nature of application	✓		
(a) New or variation	✓		
(b) Sole or joint	✓		
(c) Co-applicants	✓		
PART 2 Specific information	✓		
2.1 Details of application	✓		
(a) Nature of the novel food	✓		
(b) Proposed marketing name	✓		
(c) List of products likely to contain the novel food	✓		
PART 3 Safety Assessment considerations	✓		
3.1 Product information	✓		
(a) nature and purpose of the novel food;	✓		
(b) preparation methods /specifications, if appropriate;	✓		
(c) use overseas or by population sub-groups;	✓		
(d) stability in cooking and processing; and	✓		
(e) any requirement for processing or cooking before consumption.	✓		
3.2 Dietary intake	✓		
(a) proposed pattern of use;	✓		
(b) predicted exposure level for average and extreme consumers;	✓		
(c) predicted exposure level for any special target group.	✓		
3.3 Nutritional data	✓		
3.4 Toxicological data	✓		
PART 4 Other technical information	✓		
4.1 Energy values	✓		

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5.1 Other approvals (a) any approvals that have been granted by overseas bodies which may be relevant to the proposed use of the food. (b) whether approval has been rejected or withdrawn by any regulatory body. **5.2 Regulatory Impact Statement**	✓ ✓ ✓ ✓		
PART 6 Statutory declaration		✓	**Not applicable for overseas application (Advice from FSANZ legal officer)**

ATTACHMENT 2

Structure of PALATINIT's Novel Food Application on Isomaltulose (Palatinose™)

VOLUME I:

Main body of the submission:

Novel Food Application for Isomaltulose (Palatinose™) – (including reference list) (data file "Novel Food Application for Isomaltulose.pdf")

Annexes and References:

- **Part A: Annexes (data file "Annexes.pdf")**
  - I. Annex 1: Determination of the composition of Palatinose as such and in food using HPLC
  - II. Annex 2: Identification of Palatinose using thin layer chromatography
  - III. Annex 7: Safety Evaluation of Isomaltulose (Palatinose®). TNO Report V2575, July 2000

- **Part B: Confidential Annexes and References (data file "Confidential information.pdf")**
  - I. Annex 3: Stability of isomaltulose
  - II. Annex 4: Incubation of isomaltulose with acid
  - III. Annex 5: Studies to investigate the oral properties of isomaltulose
  - IV. Annex 6: PH telemetry tests with isomaltulose (products)
  - V. Annex 8: BfR (Bundesinstitut für Risikobewertung – Federal Institut for Risk Assessment). Bericht über die Erstprüfung von Isomaltulose (Initial Assessment Report Isomaltulose) (German + English Translation (2004)
  - VI. Annex 9: Interest of the Australian Food Manufacturing Industry
  - VII. References – **CONFIDENTIAL!:**
    - Heseker. Intake estimation in Germany (Schätzung der Verzehrsmengen von Isomaltulose) – (German / English)
    - Sydney University, Glycaemic Index Research Service (SUGiRS) (2002). Glycaemic index report – isomaltulose
    - Spengler M, Sommerauer B (1989). Tolerance and acceptance of isomaltulose (Palatinose®) compared to sucrose in a 12-week study with healthy volunteers and increasing oral doses (12-48 g). Isomaltulose Study no.101. Unpublished report by Bayer AG. 7.3.1989.

VOLUME II:

References A – J (data-file "References.pdf")

VOLUME III:

References K – Z (data-file "References.pdf"

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PART 1 GENERAL INFORMATION

1.1 Applicant

(a) Company/organisation name:
PALATINIT GmbH – Member of the SÜDZUCKER Group

(b) Address (street and postal)
Street address:
Gottlieb-Daimler Str. 12
68165 Mannheim, Germany

Postal address:
Post box 10 24 37
68024 Mannheim, Germany

(c) Contact (name(s, telephone and facsimiles numbers)

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(d) Nature of the business
Ingredient manufacturer and distributor

1.2 Nature of applicant

(a) New standard or vary an existing Standard
PALATINIT respectfully asks for an extension of the table to clause 2 of Standard 1.5.1 Novel Foods to include isomaltulose (PALATINOSE™) in column 1 of the table to clause 2.

(b) On behalf of whom is the application made

The application is made on behalf of PALATINIT GmbH, Member of the SÜDZUCKER Group, Mannheim, Germany.

(c) Co-application

No co-application is made.

PART 2 SPECIFIC INFORMATION

2.1 Details of the application

(a) Brief description of the nature of the novel food

Isomaltulose (Palatinose™) is intended to be used as a slow release carbohydrate source, in particular in those foods that contain significant amounts of carbohydrates like sucrose or other carbohydrates that are quickly absorbed to the blood stream. Isomaltulose (Palatinose™) is a nutritive low glycemic sugar.

Isomaltulose (Palatinose™, 6-O-α-D-glucopyranosyl-D-fructofuranose) is a carbohydrate (disaccharide) and like sucrose composed of glucose and fructose. In contrast to sucrose these components are linked α-1,6 glycosidic in isomaltulose instead of α-1,2 as in sucrose (see Figure 1). Isomaltulose occurs naturally in small quantities in honey and sugar cane juices. It has been used and marketed as food in Asia, mainly Japan, since 1985 and in Europe since mid 2005.

The specifications of the product intended to be marketed by PALATINIT are given in Table 2. Isomaltulose shall be used due to its combined nutritional, sensory and applicational properties as a nutritive sugar. It is soluble in contrast to long-chain carbohydrates, more stable under physiological and applicational conditions than sucrose and has a pleasant sweet taste. Its main purpose is related to its functional nutritional characteristics such as its low glycemic and low cariogenic properties. It is envisaged to use isomaltulose for its properties described above in foodstuffs in general, i.e. in beverages, cereal products, milk-based products, confectionery, bakery, marmalades, soups, dressings and desserts. It is estimated that isomaltulose might replace the use of sucrose in the market at levels of approximately 5 to 10%. An overall increase in metabolisable glucose and fructose is not expected.

To evaluate the nutritional efficacy/safety of isomaltulose numerous animal and human studies were conducted. The *in vitro* and *in vivo* data show that isomaltulose is slowly, but practically completely hydrolysed in the small intestine. The hydrolysis products glucose and fructose are absorbed and metabolised as from other sources.[1] The slower hydrolysation/digestibility in the small intestine compared to sucrose is a result of the α-1,6-glycosidic linkage between glucose and fructose. This leads to lower and slower increases in blood glucose and insulin levels in comparison with the

[1] As isomaltulose is similarly completely hydrolysed though slower than sucrose in the small intestine into glucose and fructose which are absorbed and metabolised, sucrose is the closest traditional counterpart or equivalent with a long history of safe use.

consumption of e.g. others sugars or digestible starch products. Thus, isomaltulose is low glycemic (GI 32; II 30 according to Sydney's University measurement) and would be a valuable contributor to decrease the glycemic and insulin responses which are widely discussed in relation to diabetes and preconditions, metabolic syndrome and associated diseases and also recently with obesity.

Similarly, the stability of the linkage between glucose and fructose in isomaltulose renders it more resistant to oral fermentability and therefore has a low cariogenic potential as demonstrated *in situ, in vitro* and *in vivo* studies in animals and humans.

Other nutritional data showed no health impact on tolerance, mineral absorption or lipid metabolism. Allergenicity is not expected as no protein was detected in isomaltulose and sugars themselves have not been reported to be allergenic.

In addition to the biochemical data on absorption, distribution and metabolism, the potential toxicity of isomaltulose as manufactured by Palatinit/Südzucker was studied in several subchronic (8-30 wk) and an embryotoxicity/teratogenicity study in rats as well as for mutagenicity in *Salmonella typhimurium.* Feeding doses in the pivotal subchronic and embryotoxicity/teratogenicity studies conducted at TNO amounted to 7-8 g/kg bw/day and displayed no adverse effects. The safety studies were reviewed and evaluated by TNO and it is concluded that isomaltulose is well tolerated at high levels without signs of toxicity and its use as a sugar is of no health concern.

Overall, on the basis of the nutritional and toxicological data it is thus concluded that the use of isomaltulose as a nutritive sugar poses no health concern to the consumer.

(b) The proposed name the product will be marketed under

The generic name is "Isomaltulose". The brand name is Palatinose™.

(c) An list of products that are likely to include the food

Table 1: Examples for food categories and approximate use levels (as consumed)

Illustrative examples for the food categories isomaltulose can be used in*	Examples for the description of the specific type of food within the given food category	Illustrative approx. use levels as consumed [%]
Beverages	Soft-drinks such as energy drinks sports and isotonic drinks instant drink preparations Teas Beer and related beverages Fruit or vegetable juices/drinks	1-10
Baked goods and baking mixes		10-25
Cereals and cereal products	Breakfast cereals	20-35
	Cereal bars	5-20
Soups, toppings, desserts		15-30
Milk-based products		3-20
Fruit and water ices		15
Confectionery/bakery	Hard candies	99
	Soft candies, toffees, gelees	30-50
	Chewing gum	5-35
	Chocolate and related products	25-50
	Compressed goods, tablets	98-99
	Ice creams	30
	Fondants, fillings, crèmes	90
Snack foods		10-25
Others	Jams, marmalades	25-40
	Nutritive formulae	5-20
	Energy-reduced foods	5-40
	Meal replacement/slimming food	5-20

* regular and energy-reduced

PART 3 SAFETY ASSESSMENT CONSIDERATIONS

3.1 Product information

(a) Nature and purpose of the novel food

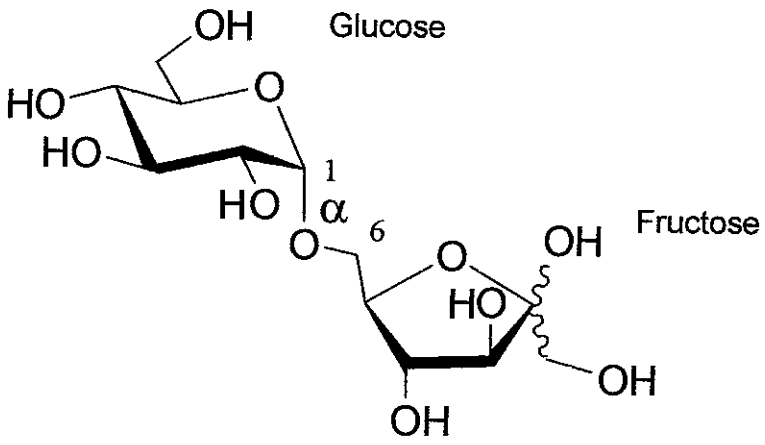
Isomaltulose is a disaccharide to be used as a carbohydrate source, replacing totally or partially sucrose or other highly digestible carbohydrates in foodstuffs. On a physical-chemical level it is similar to sucrose. From its chemical definition it is a sucrose-isomer consisting of glucose and fructose; however, compared to sucrose or glucose, it is less glycemic, less insulinemic and is less cariogenic as the linkage between glucose and fructose is more stable than in sucrose. This is leading to a slow but almost complete hydrolysis and absorption in the small intestine.

Because of its low hygroscopicity isomaltulose is used for instant powder preparations. In solution, isomaltulose offers very low inversion rates and a low tendency to hydrolyse.

(b) Specification - parameters for identity and purity of isomaltulose as produced by PALATINIT/SÜDZUCKER and analytical methods

The structural formulae of isomaltulose is given in Figure 1

Figure 1: Structural Formula of Isomaltulose



The following table (Table 2) describes the characteristics (specification) of isomaltulose produced and marketed by PALATINIT:

Table 2: Specification of PALATINOSE™

Synonyms	Palatinose™	Reference
Definition	Isomaltulose is a disaccharide derived from sucrose through the use of an immobilised enzyme preparation from *Protaminobacter rubrum* CBS 574.77	
Chemical name	6-O-α-D-glucopyranosyl-D-fructofuranose	
C.A.S No.	13718-94-0	
Chemical formula	$C_{12}H_{22}O_{11} \times H_2O$	
Chemical structure		See Figure 1
Relative molecular mass	360.3	
Assay	Not less than 98% of isomaltulose on the dry weight basis	HPLC Annex 1
Description	White or colourless, crystalline, sweet substance faint isomaltulose-specific odour	
Identification		
Solubility	Soluble in water	
Thin layer chromatography	Passes test	TLC Annex 2
Purity		
Water	max. 6 %	Karl Fischer[1]
Other saccharides	max. 2 % on the dry weight basis	HPLC Annex 1
Ash	max. 0.01 % on the dry weight basis	Conductivity[2]
Lead	max. 0.1 ppm on the dry weight basis	AAS[3]

1 Karl Fischer Method according to ICUMSA Method GS4/7/3-12 (1998)
2 Conductivity ash according to ICUMSA Method GS2/3-17 (2002)
3 Atomic Absorption Spectroscopy according to ICUMSA Method,GS2/3-24 (1998)

Material of the quality described was used in the safety studies described in later sections.

- **Typical analyses**

Several batches were analysed to validate and verify the production process. The analytical results of 5 typical batches are listed inTable 3. The data show compliance with the specification in Table 2.

Table 3: Typical analyses of five isomaltulose batches from PALATINIT/Südzucker

Batch (LIMS No.)	200311449	200311450	200311451	200311452	200311453
Water [%]	5.2	5.2	5.2	5.2	5.2
Isomaltulose [% TS*]	99.7	99.4	99.5	99.6	99.4
Total other saccharides [% TS]	0.3	0.4	0.4	0.3	0.5
Ash [% TS]	<0.01	<0.01	<0.01	<0.01	<0.01
Lead [mg/kg TS]	<0.03	<0.03	<0.03	<0.03	<0.03

* TS = Total solids

(c) Manufacturing Process

Up to now isomaltulose was an intermediate in the course of the isomalt[2]-production, a sugar alcohol (polyol) that has been marketed in Australia since 1989. Now, isomaltulose is available as a final product.

In the following text, Figure 2 and Figure 3 the production process of isomaltulose as applied by SÜDZUCKER/PALATINIT is outlined.

Isomaltulose is manufactured from food-grade sucrose. An aqueous sucrose solution passes a column with an enzyme preparation consisting of immobilised non-viable cells of *Protaminobacter rubrum* (CBS 574.77), also called 'biocatalyst'.

The enzyme responsible for the conversion of sucrose into isomaltulose is sucrose-6-glucosylmutase (EC 5.4.99.11).

The biocatalyst used for isomaltulose production is the same used in the isomalt production.
The potential pathogenicity and toxigenicity of *P. rubrum* was investigated on behalf of SÜDZUCKER with up to 10^{10} viable cells through intravenous injection in rabbits and mice followed for 14 days (Porter et al., 1991). The results showed that the viable enzyme producing organism is not pathogenic and shows only a low order of toxigenicity. The authors conclude that the *"findings provide a high degree of confidence that, if P. rubrum or its by-products should accidentally enter the final product, they will not present a hazard."*

2 In this case, the production for isomalt with a content of 1-O-α-D-glucosyl-D-mannitol (1,1-GPM) and 6-O-α-D-glucosyl-D-sorbitol (1,6-GPS) of >98 %.

SÜDZUCKER/PALATINIT uses the biocatalyst as whole non-viable cells which are immobilised by entrapment in beads of calcium alginate gel. Calcium chloride is used as coagulation agent for alginate and sodium alginate is used for matrix formation.

By the enzyme preparation, the α-1,2-linkage in sucrose is enzymatically converted into the α-1,6-linkage in isomaltulose. From the resulting solution commercial isomaltulose is obtained through crystallisation and drying. In addition, purification steps (filtration, ion exchange) are part of the process.

Figure 2: Enzymatic conversion of sucrose to isomaltulose

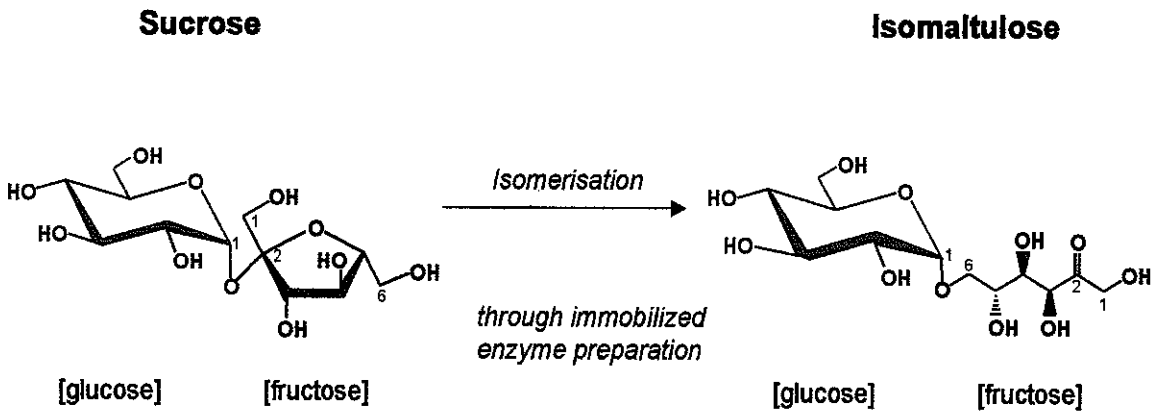
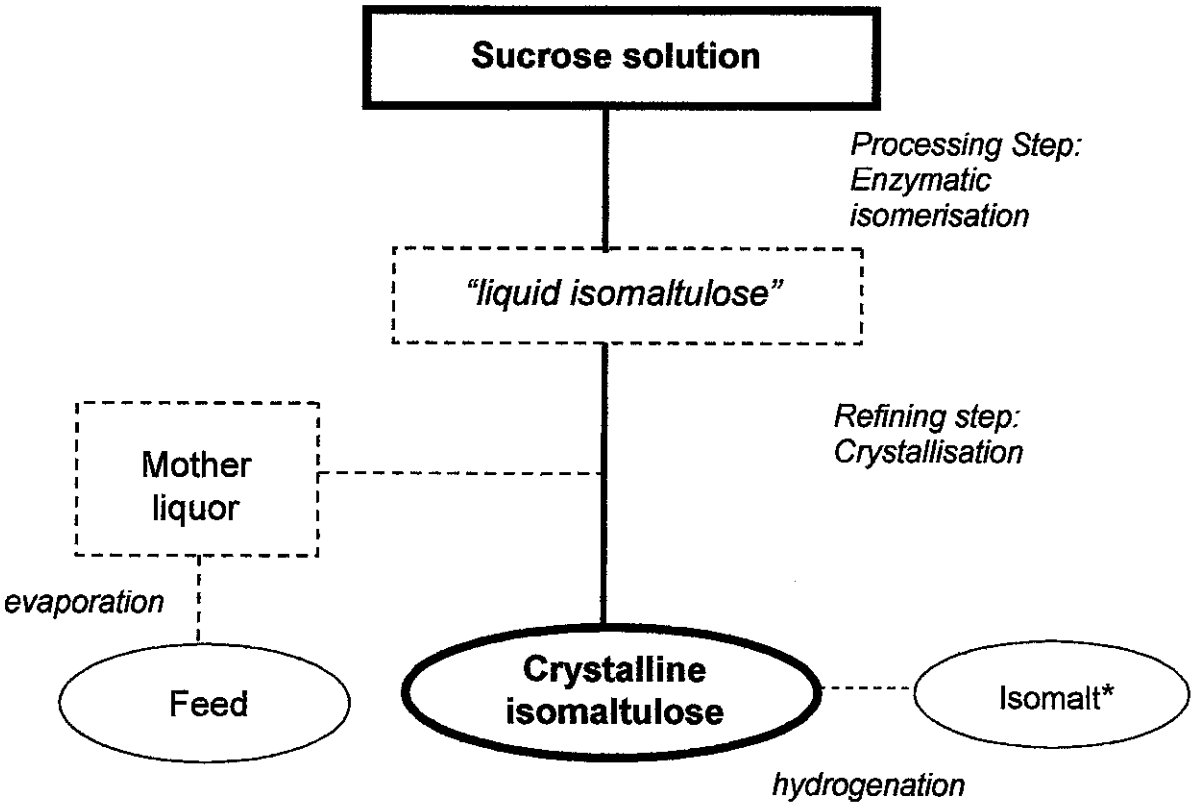


Figure 3: Production process of isomaltulose (SÜDZUCKER process)



** Content ≥ 98 % GPS + GPM*

The isomaltulose production is integrated in the SÜDZUCKER Quality Assurance System according to DIN EN ISO 9001, certified and regularly checked by an accredited body. Respective Standard Operation Procedures for raw materials, in-process as well as end product control are part of the QA system. The whole process is run in compliance with Good Manufacturing Practice as applicable to foods.
In addition to the quality assurance system, the production process was evaluated according to the principles of HACCP. This included basic processing steps, raw materials, processing aids, potential environmental and microbial contaminants as well as food contact surfaces. In conclusion, the implemented GMP measures ensure that adverse health effects from such sources can be excluded.

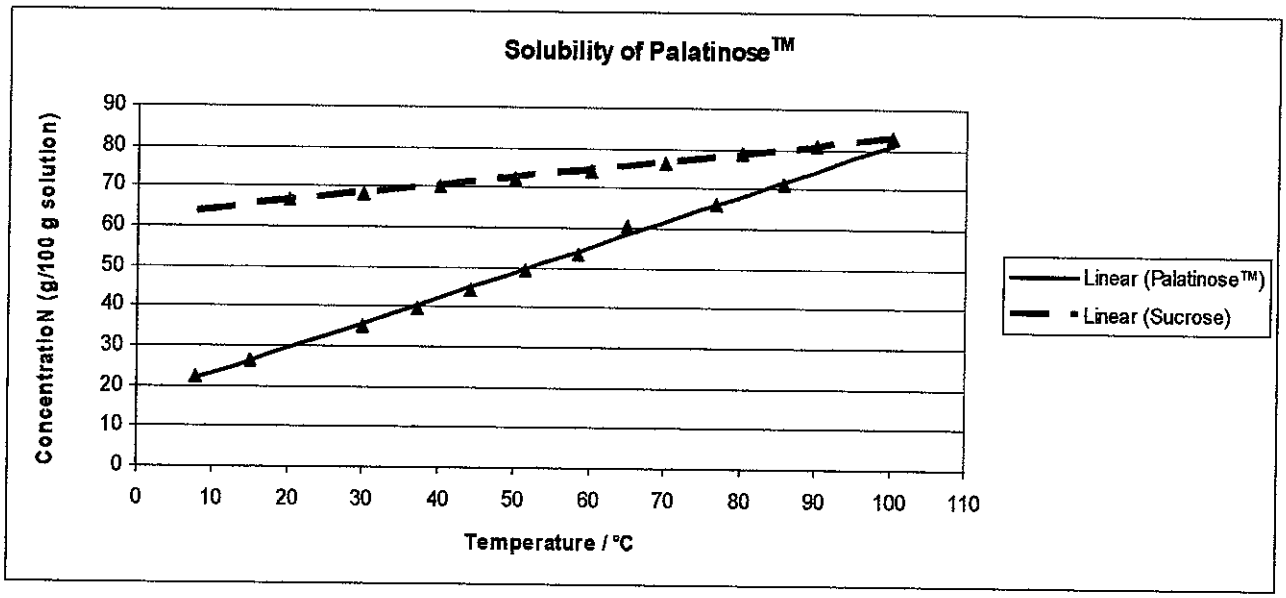
(d) Physical and chemical properties

General properties
Crystalline isomaltulose is a white substance similar to crystalline sucrose. Its melting range is about 122-124 °C and as such lower than that of sucrose (160-185 °C). Isomaltulose is practically non-hygroscopic and, unlike sucrose, remains so even on addition of citric acid (Schiweck et al., 1990; Kaga and Mizutani, 1985). The solution enthalpy of isomaltulose (-21.7 kJ/kg) is nearly the same as that of sucrose (-18.2 kJ/kg) and thus isomaltlulose does not have a cooling effect. Isomaltulose provides body/texture and mouth feeling (Schiweck et al., 1990).
The crystalline and chemical structure have been studied and confirmed by Dreissig and Lugar (1973).

Viscosity
The viscosity of isomaltulose solutions is very similar (slightly lower) to that of sucrose solutions (Irwin and Sträter, 2001).

Solubility
Isomaltulose is soluble in water (approx. 0.5 g/g water) (see Figure 4). The solubility increases with rising temperatures, reaching 85 % of that of sucrose at about 80 °C. (Irwin and Sträter, 2001; Kaga and Mitzutani, 1985).

Figure 4: Solubility of Isomaltulose versus Sucrose in Water



(e) Sensory properties

Isomaltulose provides a moderate sweetness, bulk and texture to foods.

Sweetness quality
The sweetness quality of isomaltulose is similar to sucrose; it is quickly sensed, refreshing and leaves no after-taste (Irwin and Sträter, 2001; Kaga and Mitzutani, 1985).

Sweetening power
The sweetening power of isomaltulose in comparison to a 10 % sucrose solution at 20 °C is about half that of sucrose, It rises with increasing concentrations. (Irwin and Sträter, 2001; Kaga and Mitzutani, 1985). Isomaltulose is also reported to mask off-flavors of some intense sweeteners, ingredients from fish, vegetables or soymilk (Irwin and Sträter, 2001; Anonymous 1985; Suzuki et al., 2003).

(f) Use overseas or by population sub-groups

- In Japan isomaltulose is marketed as food since 1985. Isomaltulose is marketed as well in other Asian countries (e.g., South Korea, Taiwan, China)
- In the European Union, isomaltulose was evaluated as novel food and approved as a food and food ingredient in July 2005.
- In the U.S. isomaltulose is GRAS (self affirmed). PALATINIT/SÜDZUCKER initiated as well a GRAS notification process by submitting a file to FDA in November 2005.

(g) Stability

- **Stability of isomaltulose (as such and in food, storage conditions)**
  The isomaltulose content was analysed in isomaltulose as such as well as in hard candies in intervals of two years. Data showed that isomaltulose was stable under the conditions employed (Annex 3).
- **Stability in acid solution**
  Isomaltulose is much more acid stable than sucrose. Kaga and Mizutani (1985) reported that a 20 % solution of isomaltulose at 100 °C and pH 2 did not show any degree of hydrolysis after 1 h incubation while a 20 % sucrose solution showed 95.9 % hydrolysis. At 100 °C and pH <1 complete hydrolysis of isomaltulose takes 4-5 h whereas sucrose is almost completely hydrolysed within a few minutes of boiling (Schiweck et al., 1990).
- Under physiological conditions of the stomach (37 °C, 3 h, pH 1 and 2) isomaltulose was shown to be uncleaved, too, whereas sucrose was degraded to 55 and 7 %, respectively (Annex 4).

- The high acid stability was demonstrated as well in acid food during storage over 6 month (plum liquor, pH ca. 3). While isomaltulose did not show any hydrolysis, 80 % of the sucrose was hydrolysed after 3 month (Kaga and Mizutani, 1985).

- **Heat stability**
  Isomaltulose is more heat stable than sucrose. Sucrose-based candy cooking trials lead to dark colours at temperatures >130-140°C while isomaltulose was still stable at 120°C. Comparable to other reducing sugars Maillard reaction lead to browning reactions in the presence of nitrogen compounds or condensations products may occur. (Kaga and Mizutani, 1985).

- **Bacterial stability**
  Isomaltulose is more stable than sucrose towards bacterial fermentation. This property is used in dairy products to keep their sweetness in the presence of lactic acid bacteria (Sträter and Irwin, 2001).

(h) Any requirement for processing or cooking before consumption
No requirements.

3.1 Dietary intake

While isomaltulose is suitable for consumption by the general public, its cost, formulation characteristics and metabolic characteristics will lead to the development of foods in the "healthy lifestyle" segment, marketed to consumers who follow a low glycemic diet, who are interested in avoiding significant blood sugar variations, or who (such as those engaged in athletics) are interested in a slower glucose-fructose metabolic release.

Probable Consumption of Isomaltulose
Isomaltulose is intended to be used as a slow release carbohydrate source, in particular in those foods that contain significant amounts of carbohydrates like sucrose or other carbohydrates that are quickly absorbed to the blood stream. It is the purpose of isomaltulose to replace those quickly available carbohydrates completely or partially as isomaltulose is a carbohydrate that is more slowly hydrolysed than sucrose. This leads to a lower blood glucose and insulin response. Isomaltulose provides the same calories as other carbohydrates as it is fully digestible. Further, pH-telemetry measurements showed that the oral flora can hardly use isomaltulose for their fermentation. These beneficial physiological properties will be the main reasons for using isomaltulose as a carbohydrate source.

While theoretically isomaltulose can be used at the same amount as sucrose is used, in practice, the intake level of sucrose will by far not be reached. Reasons for this are:

- Isomaltulose is significantly more expensive than sucrose is:

Isomaltulose is produced from sucrose (sugar) via enzymatic rearrangement of the α-1,2 linkage to form an α-1,6 bond. Thus, sucrose is the starting material and the complete costs of production need to be added when calculating the price of isomaltulose. Substitution of low price carbohydrates like HFCS by isomaltulose is assumed to be negligible.
Based on these significantly higher production costs, isomaltulose will be a high-price ingredient for added-value products with specific functional properties.

- Isomaltulose is less soluble in water compared to sucrose or HFCS which limits its use in high-brix syrups

- Isomaltulose is less sweet than sucrose.

Thus, it is estimated that isomaltulose might replace the use of sucrose in the market at levels of approximately 5 to 10 %.

The most up-to-date data on sweetener consumption available to us are U.S. data published in the USDA's Economic Research Service. (Haley et al. 2005). In the United States, the per capita per year consumption of total sweeteners estimated to be 46.7 kg per capita consumption (adjusted for loss), of which 21.1 kg are coming from the consumption of refined sugar while 25.3 kg are coming from corn sweeteners.

If 5-10 % of the refined sugar is replaced by isomaltulose it would lead to an intake of 1-2.1 kg isomaltulose/person/year or approx. 3 to 6 g/person/day.

In Australia, sugar use in 2001 was estimated at 50.8 kg per capita.
The Government of Australia does not publish official national consumption figures. Therefore, estimations are made based on anecdotal evidence resulting in estimated domestic sugar consumption in 2003/04 of 1.05 MMT. Based on 20,000,000 inhabitants this leads to 52.5 kg per capita per year consumption or 144g/person/day. (USDA, 2003)

Based on the assumption that isomaltulose replaces 5 to 10% of the sugar, this would result in an intake of 2.6 to 5.3 kg per capita per year or approximately 7 to 15 g/person/day.

Additional intake information:

- Isomaltulose occurs in small quantities in honey. This was first found by Siddiqui and Furgala (1967) and later confirmed by Low and Sporns (1988). It has also been reported to be in cane sugar juice (Takazoe, 1985; Eggleston and Grisham, 2003).

  Quantitative analyses of 60 samples of Spanish honeys from various regions and sources (honeydew, nectar, forest) and our own analyses from sources worldwide using gas chromatographic methods were more recently carried out (Gomez Barez et al., 2000). Isomaltulose was detected in all samples tested and ranged from 0.1 to 0.7 %.

  Average consumption of honey in the German population is estimated between 1.1 and 1.4 kg/yr (Dutch 0.65 kg, French 0.25 kg). In the US, the average consumption of honey is 1.4 pounds/yr (0.64 kg). This means that on an average basis isomaltulose is ingested in milligram to gram quantities per year in these populations (< 10 g/yr).

- In Japan isomaltulose has been marketed as food since 1985. In 2002, 5000t isomaltulose were marketed; in comparison sugar consumption amounted to more than 2 000 000 t (per capita intake 17 kg sugar in 2002).

- For Europe the sugar consumption is approx. 35 kg/yr or 100 g sugar/person//day).
  An estimation of the intake of isomaltulose in Germany was based on a 3 tier approach: estimation based on the total usage of sugars per person, estimation of the possible contribution of particular foodstuffs and estimation based on consumption surveys. It was assumed isomaltulose can replace 5-10% of the total sugar consumption by use of the relevant products. 20% was used as a maximum calculation. This resulted in the estimation of a daily average intake of isomaltulose of 15-35 g, assuming the alternative food product containing isomaltulose were chosen from the food selection at each opportunity. A particularly high level of consumption could, in an extreme case, possibly lead to a daily intake of up to approximately 100 g. All calculations are to be regarded as theoretical maximum amounts. The anticipated mean isomaltulose intake would probably be significantly lower in reality."(Heseker, 2004)

3.2 Nutritional data

Isomaltulose is a disaccharide sugar (carbohydrate) and as sucrose composed of glucose and fructose. Isomaltulose is hydrolysed in the small intestine into its components which are absorbed and metabolised as derived from sucrose. The hydrolysis is virtually complete; therefore, the physiological energy value also corresponds to that of sucrose (4 kcal or 17 kJ).

Compared to sucrose, however, the rate of hydrolysis of isomaltulose is much lower resulting in a much lower glycemic and insulinemic response. Isomaltulose is therefore considered as 'low glycemic' (Sydney University, 2002) whereas the overall intake of metabolisable glucose and fructose remains constant.

Similarly, the stability of the linkage between glucose and fructose in isomaltulose renders it more resistant to oral fermentability.

In view of healthier diets these functional properties of isomaltulose are the main reason for its intended use instead of rapidly digestible and orally fermentable carbohydrates as ingredient in foods.

- **Low glycemic properties**

There is an increasing scientific debate about glycemic properties and benefits of low glycemic foods/diets for health and metabolic diseases (e.g. Brand-Miller, 2003). Health effects of low glycemic diets are widely discussed in relation to

- Dietary management of diabetes and potential predispositions
- Decreasing risk in developing metabolically-based non-communicable diseases including insulin resistance, syndrome X, diabetes or cardiovascular diseases
- Weight control/obesity.

Many nutritionists see an increasing need to reduce the glycemic index (GI) of foods[3]. Other discussed important parameters include the glycemic load (GL=GI multiplied by carbohydrate content), or postprandial glycemic peaks. In any case, low glycemic foods become increasingly important for healthier diets (FAO/WHO, 1998). Typically, the GI concept focuses on digestible and metabolisable carbohydrates causing a lower blood glucose response.

The effect of isomaltulose on blood glucose and insulin levels was studied in healthy and diabetic humans (Macdonald and Daniel, 1983; Kawai et al., 1985, 1989; NutriScience 2002, Hespel et al., 2003; Achten et al., 2003; Liao et al., 2001; Sydney University, Glycaemic Index Research Service (SUGiRS), 2002).

Orientating studies with healthy subjects were conducted with up to 0.5 g isomaltulose or sucrose per kg bw (Macdonald and Daniel, 1983). Blood glucose levels and insulin levels increased more slowly and reached slower peaks with

[3] The GI is defined as the incremental area (IAUC) under the blood glucose response curve of a 50 g carbohydrate portion of a test food as percentage of a standard food (glucose or white bread) (for details FAO/WHO 1998).

isomaltulose than with sucrose; serum insulin and fructose levels were only half with isomaltulose. In this study the areas under the curve for glucose were comparable.

Studies conducted by Kawai (1985, 1989) with healthy and diabetic subjects showed that increases in plasma glucose and insulin (peaks and IAUC) following oral administration of 50 g isomaltulose are significantly smaller than those seen after sucrose. (for review see Lina and Woutersen, 2000; Lina et al., 2002).

GI and II (insulinemic index) determinations of isomaltulose according to generally accepted international standards were carried out with 10 healthy subjects who received 50 g of test carbohydrates (Sydney University (SUGiRS), 2002). Blood glucose and insulin levels were followed for 120 min. The GI and II of isomaltulose were 32 and 30, respectively, with glucose as the reference food. The authors confirmed the low GI and low glycemic properties of isomaltulose as a digestible and metabolisable sugar. Only, the values of fructose were lower (11 and 15, respectively). This is consistent with observations reported by Hespel et al., 2003, who compared blood glucose and insulin response to isomaltulose and fructose before and after exercise in insulin resistant subjects.

GI and II values of 56 and 48 for isomaltulose (glucose = 100) are reported in Cargill 2003 (NutriScience 2003) in a study not following generally accepted methodology (doses of approx. 80 g were administered and followed for 4 h). For a 50 g intake Suzuki et al., 2003, determined 44 in comparison to glucose. Lower glycemic and insulinemic changes were also reported by Liao et al., 2001 who administered 75 g of isomaltulose or sucrose to healthy and diabetic subjects. Significant effects on blood lipids were not observed.

In total the data confirm that isomaltulose is more slowly digested than sucrose and leads to respective attenuated blood glucose and insulinemic responses. These low glycemic properties make it a useful metabolisable sugar that helps to reduce negative health effects associated with high glycemic diets.

- **Low cariogenic properties**

General
Dental caries is a multifactorial disease, but mainly related to acid formation from fermentable carbohydrates such as glucose, sucrose, lactose or starches through oral plaque bacteria (e.g. mutans streptococci, other oral lactobacilli, actinomycetes) at the tooth surface. An intra-oral pH drop below 5.7 *in vivo* is regarded as critical for tooth demineralisation and tooth decay (Gehring, 1973; Imfeld, 1983; Birkhed et al., 1985; Hamada, 2002).

A second critical etiological factor is the formation of insoluble, adhesive glucan polymers (e. g. from sucrose or starchy foods) which are the prerequisite to provide an habitat for cariogenic micro organisms at the tooth surface (Hamada, 2002).[4]

Studies with isomaltulose
With isomaltulose various study-types to assess the oral properties were conducted. They comprise essentially

- *in vitro* fermentation studies with pure and mixed cultures of known cariogenic bacteria or plaque to study acid formation
- *in vitro* and *in vivo* experiments on the influence of isomaltulose on glucan syntheses
- *in vitro* experiments on the effect of isomaltulose on demineralisation
- animal studies on the cariogenic potential of isomaltulose
- *in situ* pH telemetry studies in humans for intraoral acidogenicity

Relevant studies are summarised below and have been reviewed in detail by Birkhed et al. (1985, 1987), Siebert et al. (1988), Takazoe (1985, 1989) and Hamada (2002). References for the aspects described below are listed in Annex 5:

- Acid production from isomaltulose (*in vitro*)
  The ability of various strains of mutans streptococci and other oral bacteria to ferment isomaltulose has been tested in several *in vitro* studies. Most strains, in particular mutans streptococci, failed to grow in culture media containing isomaltulose or grew very slowly, acid production was usually less compared to highly fermentable sugars such as glucose or sucrose used as positive controls apart from some studies with very long incubation time (more than 24 hrs). It has also been shown, using *in vitro* suspensions of dental plaque containing mixed flora from humans, that acid production or lactate production by isomaltulose was considerably lower than that produced with sucrose or glucose.

- Effect of isomaltulose on glucan synthesis (*in vitro*)
  Several *in vitro* studies suggest that isomaltulose may have the potential to inhibit the synthesis of insoluble glucans from sucrose by mutans streptococci. A proposed mechanism is that isomaltulose may function as acceptor for glucose to form soluble oligosaccharides rather than insoluble plaque-forming polysaccharides. No water-insoluble glucan was synthesised from isomaltulose alone by *S. mutans*.

- Effect of isomaltulose on demineralisation (*in vitro*)
  In an *in vitro* design isomaltulose did not show significant effects on demineralisation when exposed alone. However, it did similar to other carbohydrates tested enhance the demineralising effect of fluoride.

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4 Other aspects discussed but with yet unclear relevance in prevention of overt caries are among others inhibition of demineralisation, enhancement of remineralisation and anti-microbial features.

- Experimental animal studies on isomaltulose (*in vivo*)
  With isomaltulose, animal (rat) studies were carried out, too. Rats were typically superinfected with oral pathogenic bacteria and received either isomaltulose, starch, glucose, fructose, sucrose or mixtures thereof. In these experiments, caries, partially the severity, plaque and/or cell counts of oral pathogens were significantly lower with isomaltulose than with sucrose. Even supplementation of sucrose by isomaltulose usually decreased dental decay or risk factors.

- Human studies on isomaltulose (*in situ*)
  The intra-oral plaque pH-telemetry is a well established and accepted method to investigate *in situ* in humans whether a product can be classified as not promoting tooth decay. The basic principle of the method is to measure acid production from fermentation of carbohydrates directly in a plaque at the surface of the tooth according to an internationally accepted protocol. The measurement is carried out with a plaque covered electrode which is fixed in a bridge in the mouth of a volunteer. As the ‚critical' fermentation limit, pH 5.7 has been fixed. If the pH drops below 5.7 the product has a significant acidogenic potential and is thus considered cariogenic. As a positive control an easily fermentable sugar, typically sucrose, is used, for which the pH should drop down below pH 5.0. (Imfeld (1983).

  Some profiles of isomaltulose/sucrose rinses as well as isomaltulose candies and teas are shown in Annex 6.

  Several modified intra-oral and/or plaque pH measurements were carried out. The results were consistent with the standard pH telemetry studies: isomaltulose was found to be less acidogenic compared to conventional sugars such as sucrose, lactose or fructose (see reference list in Annex 5).

- Other studies in humans
  Several other studies for the oral properties of isomaltulose were carried out in humans. Among others it was found that isomaltulose leads to lower plaque formation compared to sucrose. Similar results were obtained when isomaltulose-based snacks or coffee sweetened with it were supplied for 3 days.

Overall, the available data show that isomaltulose does lead to lower acid formation *in vitro* and *in vivo* than easily fermentable sugars. It does not lead to glucan formation itself and data suggest that it may inhibit the glucan synthesis from sucrose. Experiments in animals and pH-telemetry studies with human volunteers also indicate that isomaltulose has a low cariogenic risk. Thus, isomaltulose can be considered low cariogenic in comparison to traditional saccharides.

- **Other nutritional aspects**

- Effects on mineral absorption
  No effect of isomaltulose on mineral absorption such as Ca, Mg, P and others was found in animal studies (Kashimura et al., 1990a; Kashimura et al, 1996; see also Annex 7)

- Effects on lipid metabolism
  Animal studies on acute and long-term effects of isomaltulose on triglycerides, free fatty acids and liver lipids after oral or intravenous administration were carried out in rats and dogs (Suzuki et al., 1992; Kawai et al., 1986). The parameters analysed were not significantly changed (see Annex 7).

  Blood lipid parameters were side parameters in studies aimed at other issues with healthy and diabetic subjects. They included LDL, VLDL, HDL-C, total cholesterol and triglycerides. No differences were observed due to isomaltulose compared to pre-test or control substance values apart from a slight increase in triglycerides in one study which was still very well in a normal range and considered incidental (Kashimura et al., 1990b, Liao et al., 2001; Lina and Woutersen, 2000).

- Physical exercise
  One study was conducted to assess effects of isomaltulose in exercise (cited in Cargill 2003). It is reported that half the energy is derived from isomaltulose oxidation compared to sucrose oxidation, i. e. suggesting a higher utilisation of endogenous glycogen and fat with isomaltulose (Achten et al., 2003).

- Mental performance
  Preliminary investigations were made on effects of isomaltulose on mental concentration in humans in comparison to sucrose (Kashimura et al., 2003). The data indicate a similar, but longer lasting effect on concentration than sucrose.

- Prebiotic aspects
  Isomaltulose does not reach the colon to a significant extent and it is expected that it will not function as a colonic prebiotic. Consistent with this assumption, even with 24 g isomaltulose/day no influence on the absolute and relative composition of faecal microflora was observed (Kashimura et al., 1990b).

  In vitro experiments showed, however, that it was metabolised by most bifidobacteria and lactobacilli tested in contrast to typical peptolytic gut bacteria (Kashimura et al., 1991; Mitsuoka, 2002). This could be a promising property as stabiliser of probiotic cultures added to food.

Nutritional impact

Isomaltulose is intended to be used mainly for its low-glycemic and/or tooth-friendly properties in order to replace higher glycemic and/or cariogenic carbohydrates (e.g. glucose, fructose, sucrose, starch hydrolysates). From the uses envisaged and the nutritional properties, like for these digestible carbohydrates negative health effects for isomaltulose can be excluded.

3.3 Toxicological Data / Safety Evaluation

Isomaltulose is like sucrose composed of glucose and fructose, linked α-1,6 instead of α-1,2 (see Figure 1). Isomaltulose is slower, but according to the available data (see below) almost completely hydrolysed to glucose and fructose in the small intestine which are absorbed and metabolised like glucose or sucrose from traditional sources. In this regard, isomaltulose and its constituents can be considered equivalent to glucose, fructose, sucrose as well as to a certain extent to digestible starch and its constituents (glucose moiety, only).

The available biological data were reviewed and evaluated by independent toxicologists on the request of SÜDZUCKER. The expert's safety evaluation is provided in Annex 7 (Lina and Woutersen, 2000; Lina et al., 2002). In the following only key and additional issues are summarised.

Biochemical data: absorption, distribution, metabolism and excretion

- **General**

Similar to disaccharides such as sucrose, maltose and isomaltose, isomaltulose is hydrolysed by the disaccharidase sucrase/isomaltase into its constituents glucose and fructose (Heinz, 1987; Cheetham, 1982).The sucrase/isomaltase complex is located in the brush border membrane of the mucosal cells lining the lumen of the small intestine with highest activity in the jejunum in humans (Cheetham, 1982). It is an aggregate of two separate peptides with differing substrate specificities. Isomaltulose is hydrolysed by the isomaltase site of the sucrase-isomaltase complex. The resulting glucose and fructose moieties are absorbed into the systemic circulation via active and passive transport systems. Whilst isomaltulose is almost completely hydrolysed by the enzyme in the mucosal cells, its rate of hydrolysis is slower than that of sucrose, with a correspondingly slower rate of systemic absorption of glucose and fructose.

Several *in vitro* and *in vivo* studies on hydrolysis of isomaltulose, in comparison with that of other disaccharides, and absorption of the component monosaccharides have been conducted in rats, rabbits, pigs, dogs and humans (see Annex 7 and Cheetham, 1982, for review and older anecdotal data).

- ***In vitro* studies**

In vitro studies were conducted with small intestinal homogenates from rats, pigs and humans as well as purified enzyme preparations from rats, rabbits and humans. The data showed that isomaltulose is hydrolysed by the sucrase/isomaltase complex but to a much slower rate than sucrose or maltose (approx. 10-20%, occasionally higher, see Table 4).

From the *in vitro* experiments it was also possible to identify that in humans isomaltulose is hydrolysed by the isomaltase subunit, whereas sucrose is cleaved by the sucrase peptide (e. g. selective inhibition of isomaltose but not sucrose hydrolysis, selective heat inactivation etc.); (Heymann and Heinz, 1987; Günther and Heymann, 1998).

Table 4: **Rates of hydrolysis of isomaltulose compared with sucrose and maltose by small intestinal preparations in vitro**

Species	Rate of hydrolysis of isomaltulose (%)		Reference
	Versus sucrose	Versus maltose	
Pig	10 – 20	2 – 5	Dahlqvist, 1961
Pig	5.6	4.7	Heinz, 1987
Rat	11.8	4.4	Yamada et al., 1985
Rat	11.4	2.4	Tsuji et al., 1986
Rat	1.8	3.7	Heinz, 1987
Human	44.7	11	Grupp and Siebert, 1978
Human	12.7	9	Ziesenitz, 1986a
Human	26*	8*	Ziesenitz, 1986b
Human	16	18	Heinz, 1987

* 5 Sugars added in a mixed preparation; all other studies sugars added individually

- **Intravenous *in vivo* studies**

Disaccharides are virtually not passing the intestinal wall as such apart from minute amounts (Menzies, 1974). The potential systemic fate of isomaltulose, if any, has been investigated following intravenous administration in rats, dogs and humans. In rats and dogs, increases in plasma glucose and insulin levels indicate hydrolysis by tissues such as the liver (Okuda et al., 1986; Kawai et al., 1986; Hall and Batt, 1996). Studies in humans using a variety of sugars, including sucrose, lactose and isomaltulose, given intravenously, have shown that most of the administered sugar is excreted unchanged in the urine within the first 2.5 hours (Menzies, 1974). These results suggest that in man hydrolysis by tissues other than the intestinal mucosa is minimal.

- **Oral *In vivo* studies**

Oral *in vivo* biochemical studies were conducted in rats, pigs, dogs, healthy and diabetic humans.

Administration of radio labelled isomaltulose and sucrose to rats yielded comparable recovery rates from urine, faeces and breath with both substances (total recovery c. 60%); the major portion of radioactivity was found in exhaled air within the first hours of intake. Only minor amounts of radioactivity were found in urine or faeces. The data indicate that isomaltulose is digested, systemically absorbed and metabolised in principle comparable to sucrose.

Studies with fistulated pigs showed that isomaltulose given at 20 % in the diet is almost completely digested in the small intestine. In regard to the control substance sucrose slightly higher fructose levels were detected in the chyme. Differences in the amounts of fermentation products (acids) could not be detected in the chyme. The results show that both sugars are hydrolysed and absorbed in the small intestine; however, isomaltulose is less rapidly digested than sucrose. The flow of wet ileal chyme was almost identical in both groups, demonstrating good tolerance and no increased osmotic effect of the diet containing 20 % isomaltulose (van Weerden et al, 1983).

An ileostomy study was conducted in humans: On average, it is reported that more than 90 % (85-96%) of a high 75-g-isomaltulose bolus disappeared in the small intestine (NutriScience, 2003). These results are consistent with the data from pigs.

Several studies were conducted in dogs, healthy and diabetic humans (Macdonald and Daniel, 1983; Kawai et al., 1985, 1986, 1989; NutriScience 2002, Hespel et al., 2003; Achten et al., 2003; Liao et al., 2001; Sydney University, 2002) with the main objective to investigate blood glucose and insulin response (see 3.2). In a recent study the Glycemic Index (GI) of isomaltulose was determined following an up-to-date procedure (Sydney University, 2002). For isomaltulose a GI of 32±3 was determined with glucose as the reference food of a GI 100 (for fructose, sucrose and maltodextrin 11±1, 68±4 and 86±8 respectively). The insulin index (II) value corresponded to the GI values (II value of Isomaltulose 30±5). Fullness responses were comparable.

In summary, the studies showed that isomaltulose compared to sucrose leads to a slower and lower glucose, fructose and insulin response, but that isomaltulose is hydrolysed into its constituents and is almost completely absorbed during transit in the small intestine in man as well as in other species. The studies also indicate that isomaltulose could be used in place of high-glycemic sweeteners to produce processed food with lower GI and II values for healthy as well as for diabetics.

Toxicological data

Isomaltulose was studied in several subchronic (8-30 wk) and an embryotoxicity / teratogenicity study in rats as well as for mutagenicity in *Salmonella typhimurium*.[5] The material used originated from the production processes applied by Südzucker and Mitsui; for the 90-day study (Jonker et al., 2002) the crystallisation step (see Figure 3) was deliberately reduced in order to have potential unknown contaminants, if any, to the highest feasible extent. Feeding doses in the pivotal subchronic and embryotoxicity/teratogenicity studies conducted at TNO amounted to 7-8 g/kg bw/day.

These studies were evaluated and summarised (Lina and Woutersen, 2000, Annex 7; Lina et al., 2002).

The authors concluded after detailed and critical consideration of the results reported that
"Isomaltulose was non-mutagenic in the Ames test and did neither induce maternal toxicity nor embryotoxic or teratogenic effects in rats. Oral administration of large doses of isomaltulose in sub-chronic toxicity studies did not reveal signs of toxicity. This was confirmed in a recent OECD and FDA compliant feeding study, showing no adverse effects up to the highest dietary level (10%) tested. Overall it can be concluded that isomaltulose is well tolerated at high levels without signs of toxicity."

Specific data on tolerance

As stated in the previous sections, isomaltulose is hydrolysed in the small intestine and the resulting monosaccharides glucose and fructose are absorbed and metabolised. Thus, laxative effects, which are sometimes seen with low-digestible carbohydrates are generally not expected; neither are effects on the colonic flora.

A rare disorder in humans is sucrase-isomaltase deficiency either congenital or associated with brush border dystrophies. Affected people are not able to digest sucrose completely. Consequently, sucrose reaches the colon and is fermented similar to soluble fibre or lactose in lactase-deficient people. Watery stools may occur. It is reported that isomaltulose is used as a diagnostic. Thus, sucrose-intolerant subjects may also not be able to digest isomaltulose. However, this intolerance is much less common than lactase deficiency (further reading in Auricchio et al., 1963; Burgess et al., 1964; Cheetham, 1982; Dawson, 1970; Zellow and Saavedra, 1999).

Gastrointestinal tolerance data are available from *in vivo* studies in rats, pigs and humans (most studies reviewed in Lina and Woutersen, 2000; Lina et al., 2002).

In rats, in studies with administration of even 56 % via the diet or 0.5 g/kg bw no diarrhoea or soft stools were observed (Macdonald and Daniel, 1983; Takazoe et al.,

[5] Allergenicity is not expected as no protein was detected in isomaltulose. Sugars themselves have not been reported to be allergenic.

1985; Topitsoglou et al., 1984; Sasaki et al., 1985; Ziesenitz, 1988). Isomaltulose given to pigs for 14 days at 20% (approx. 16 g/kg bw) in the diet was as well tolerated as was sucrose (van Weerden et al., 1983). In none of the toxicity studies diarrhoea was reported.

Several studies in humans confirmed acceptance and gastrointestinal tolerance. In studies with single doses focussing essentially on glucose and insulin metabolism, doses up to 50 g or 1 g/kg bw were administered and did not result in gastrointestinal discomforts or even diarrhoea (Kawai et al., 1985; 1989; Macdonald and Daniel, 1983; Hespel et al., 2003)

Longer-term studies specifically aimed for tolerance in healthy adults were conducted up to 12 weeks by Spengler and Sommerauer, 1989, and Kashimura et al., 1990b. Daily intake amounted to 50 g through various food products. In conclusion, isomaltulose was similarly tolerated and accepted as sucrose.

Summarising, inherent safety of isomaltulose can be inferred from the knowledge that it is hydrolysed in the intestinal mucosa to the nutrients glucose and fructose, which are then absorbed and metabolised as from other dietary sources. Furthermore, isomaltulose as such is not systemically absorbed unless in very small amounts. Its safety, therefore, can be considered as similar to that of sucrose, glucose and fructose or starch hydrolysates. No adverse reactions would be expected other than intolerance in persons with rare sucrase-isomaltase deficiency. This risk can be managed by adequate labelling, such as the listing of isomaltulose in the ingredient list, as is the case for sucrose (sugar). No allergenic risk to humans is anticipated from a disaccharide (origin: sugar from sugar beets).

PART 4 OTHER TECHNICAL INFORMATION

4.1 Energy values

Isomaltulose is almost fully hydrolysed and absorbed in the small intestine. Therefore the same energy factor as used for other available carbohydrates should be used for isomaltulose (17 kJ/g).

PART 5 REGULATORY/LEGISLATIVE IMPLICATIONS

5.1 Other approvals

Isomaltulose is marketed as a sugar (food) in a number of Asian countries for many years now (Japan, South Korea, Taiwan). In China, isomaltulose is approved food addtitive as at the time of approval (prior 1996) this was the only way to get something approved by the Ministry of Health. In Japan, isomaltulose (Palatinose) is even included in the FOSHU regulation.

In Europe Isomaltulose is regarded as food as well. This classification was the result of a novel food approval process that was initiated by SÜDZUCKER/PALATINIT by submitting a file to the German Health Authorities for their initial safety assessment. Annex 8). This assessment of the German Authorities and the complete file of SÜDZUCKER/PALATINIT were given to the EU Commission who shared it with all member states. The process resulted in the approval of isomaltulose produced by SÜDZUCKER/PALATINIT as a novel food or novel food ingredient for use in foodstuffs. (Commission Decision of 25 July 2005 authorising the placing on the market of isomaltulose as a novel food or novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and the Council (2005/581/EC). Official Journal of the European Union L199/90, 29.7.2005)

In the United States of America, isomaltulose (Palatinose™) has been determined to be Generally Recognized As Safe for use in food as a carbohydrates source replacing totally or partially sucrose or other highly digestible carbohydrates. This determination has been made by SÜDZUCKER/PALATINIT based upon scientific procedures and has resulted in the determination by experts that the substance is Generally Recognized As Safe. A written notification of the GRAS status of isomaltulose was provided to FDA by SÜDZUCKER/PALATINIT in late October 2005. FDA is currently reviewing the data.

No approval was rejected or withdrawn by any regulatory body.

5.2 Regulatory Impact Statement

Consequences of an approval of the use of isomaltulose in Australia and New Zealand:

- Manufacturers of food products in Australia and New Zealand will be enabled to produce high quality and excellent testing foods and drinks with low(er) GI values (contribution to a healthy lifestyle). The interest in this ingredient in the Australian Food industry is indeed large as expressed in Annex 9.
- Consumers who are interested in a low glycemic diet will be served by food manufacturers.

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Annex 9 Interest of the Australian Food Manufacturing Industry

PART A – Annexes

(data file "Annexes.pdf")

ANNEX 1:

Determination of the composition of Palatinose as such and in food using HPLC

SÜDZUCKER Standard Operation Procedure Doc-No SZ/A-10.P37, Version 09-24-2001

Determination of the Composition of Palatinose *as such and in food* using HPLC

1. **Place of Application**

   ZAFES

   Isomalt laboratory

2. **Responsibility for Performance**

   Coworkers in the laboratory

3. **Supporting Documents**

Doc.-No.	Title	Filing
	Instrument manuals	Laboratory

elaborated:	in conformity with quality system	checked and released	enforced
date: 28.09.01	date: 28.09.01	date: 01.10.2001	date: 02.10.2001
sign: Martin	sign: JmR	sign: Pulte	~~sign:~~

Determination of the Composition of Palatinose *as such and in food* using HPLC

4. Determination

4.1 Definition

Object of this procedure is the quantitative determination of palatinose (isomaltulose) and accompanying saccharides such as sucrose, trehalulose, isomaltose, glucose and fructose in crystalline palatinose or in solutions containing palatinose, respectively. *The sample preparation for the determination of palatinose in food is described under 4.6.*

The contents of these saccharides are expressed in g/100g dry substance.

4.2 Abstract / Principle

Sample solutions are analyzed by separation on an amino bonded silica gel column with an acetonitrile/water eluent using a HPLC apparatus.

The resolved components leaving the column are detected using a differential refractometer and quantified by comparison of the peak areas with those of an external standard.

4.3 Reagents / Material

4.3.1 Eluent

- Purified water, HPLC grade (deionized, double-distilled, filtered through a 0.22-µm membrane)
- Acetonitrile, HPLC grade (e.g. Merck, No. 114291)

To prepare a 71% solution of acetonitrile, 3550 ml of acetonitrile are mixed with 1450 ml of purified water in a 5 l-bottle and degased with helium for 30 min. If necessary, the amount of acetonitrile can be increased up to 74 %.

4.3.2. Standard solution

- Isomaltulose (Palatinose®) crystallizes with 1 mole of water (water content approx. 5%)
- Trehalulose
- Isomaltose
- Sucrose
- Glucose

Determination of the Composition of Palatinose *as such and in food* using HPLC

- Fructose

Water contents of all standard substances are exactly determined using the Karl-Fischer method.

Furthermore each of the standard substances is analyzed separately under the conditions described in this procedure in order to detect impurities.

Water contents and purities of the standard substances must be considered in the calculation.

The following amounts of standard substances (calculated as pure dry substance) are weighed into a balance dish to an accuracy of ± 0.1 mg:

	For the calibration of samples with high concentrations of palatinose (e.g. crystalline palatinose)	For the calibration of samples with low concentrations of palatinose (e.g. tests of enzyme activity)
isomaltulose (Palatinose®)	approx. 9.85 g	approx. 1.00g
trehalulose	approx. 0.06 g	approx. 0.40 g
isomaltose	approx. 0.06 g	approx. 0.40 g
sucrose	approx. 0.01 g	approx. 7.50 g
glucose	approx. 0.01 g	approx. 0.50 g
fructose	approx.0.01 g	approx.0.50 g

The standard substances are transferred quantitatively into a 100-ml graduated flask, dissolved in purified water, tempered to 20°C and filled up to the mark.

The standard solution can be stored deep frozen in portions of 1ml for up to one year. The concentrations of the standard substances calculated with consideration of impurities and water contents have to be reported.

Determination of the Composition of Palatinose *as such and in food* using HPLC

4.4 Equipment / Auxiliary Devices

HPLC-instrument containing:

- HPLC-pump
- autosampler
- RI detector
- precolumn: 10 x 4.6 mm, amino bonded silica (e.g. Zorbax-NH_2)
- separating column: 250 x 4.6 mm, amino bonded silica (e.g. Zorbax-NH_2)
- protection column (used separating column for the fitration of the eluent)
- interface, computer and software for date recording and evaluation

for the preparation of samples and standards:

- analytical balance, reading precision ± 0.1 mg
- spoon for chemicals
- 100-ml graduated flasks
- 1000-µl pipette (e.g. Eppendorf) with tips
- vials with screw cap (e.g. N 8-1, Macherey - Nagel)
- thermostat, operating temperature: 20°C
- *membrane filter (0.45µm)*

for the eluent:

- magnetic stirrer
- magnetic stirring bar
- 5-l glass bottle
- refractometer

4.5 Preparation of Measurement

Instrument parameters:

- injection volume: approx. 10µl (dependent on the sensitivity of the applied RI-detector)
- flow rate: approx. 1.3 - 1.8 ml/min

Determination of the Composition of Palatinose *as such and in food* using HPLC

4.6 Sample preparation

Samples of approx. 10 g of crystalline palatinose are dissolved in purified water and diluted to a volume of 100 ml at 20°C.

Samples of palatinose containing solutions are diluted with purified water, to obtain a concentration of approx. 10% dry substance (refractometer).

Hard candies or instant teas containing palatinose are dissolved with purified water. The dry substance content of the sample solution should be approx. 10%.

Turbid solutions are filtered.

Incompletely soluble samples, such as pastries or chocolate are extracted with purified water. Therefore weigh approx. 25 g of the homogenized sample in a 100 ml volumetric flask and mix with 50 ml of water, stir for 60 min at ambient temperature (e.g. pastries) or at 40 – 60°C (chocolate) and dilute to the mark. When the precipitate has settled, filter the aqueous phase through a membrane filter (4.4). Alternatively the sample solution may be centrifuged . Fat containing sample solutions sometimes need to be filtered a second time through a membrane filter of smaller pore size.

4.7 Procedure

- Vials filled with standard and sample solutions are placed into the autosampler
- Preparation of the evaluation program: load corresponding method file
- start autosampler

4.8 Calculation

Quantitative analysis of the peak area measurement is carried out according to the '100 %-method'. Therefore the response factors of the palatinose, sucrose, trehalulose, isomaltose, glucose and fructose are calculated by means of external standards. If there are others than the above mentioned substances present in the chromatogram, the calibration factor of palatinose is applied for their calculation. The contents of all detected components are summarized and the sum is assumed to represent 100 %. Response factor f_A of component A:

Determination of the Composition of Palatinose *as such and in food* using HPLC

$f_A = a_A/m_A$

a_A = peak area of component A in the calibration solution (standard solution)

m_A = mass of component A in the injection volume

Analysis of the sample solution X gives the peak area of component A. The mass of component A is calculated by the following formula:

$m_{X,A} = a_{X,A}/f_A$

The calculated mass of component A is converted into its content in the sample:

$w_{X,A} = 100 * m_{X,A} / \Sigma m_{X,i}$

The content of component A in the sample is given in % resp. in g/100 g dry substance.

This calculation is carried out with a suited evaluation program (e. g. Chemstation).

The retention times of the components are given by the chromatograms in the appendix; they can vary depending on different flow rate, performance of the column and composition of eluent. Approximate values for the standard conditions (flow rate: 1.4 ml/min; acetonitrile: 71 %) are as follows (± 0.5 min):

Isomaltulose:	8.4 min
Trehalulose:	9.4 min
Isomaltose:	10.2 min
Sucrose:	7.7 min
Glucose:	6.6 min
Fructose:	5.5 min

Determination of the Composition of Palatinose *as such and in food* using HPLC

4.9 Validation

If complete separation between palatinose and trehalulose can not be achieved using this method, the peak area of the trehalulose peak should not be determined by perpendicular but by tangential peak separation (prolongation of the palatinose peak).

Thus the obtained results can be confirmed by other methods (e.g. capillary gas chromatograpy).

4.10 Report

Chromatograms are collected in the corresponding document file.

The contents of each of the components are expressed in g/100g dry substance (to an accuracy of ±0.1 g/100 d.s.) and stored in a data file.

5. Appendix

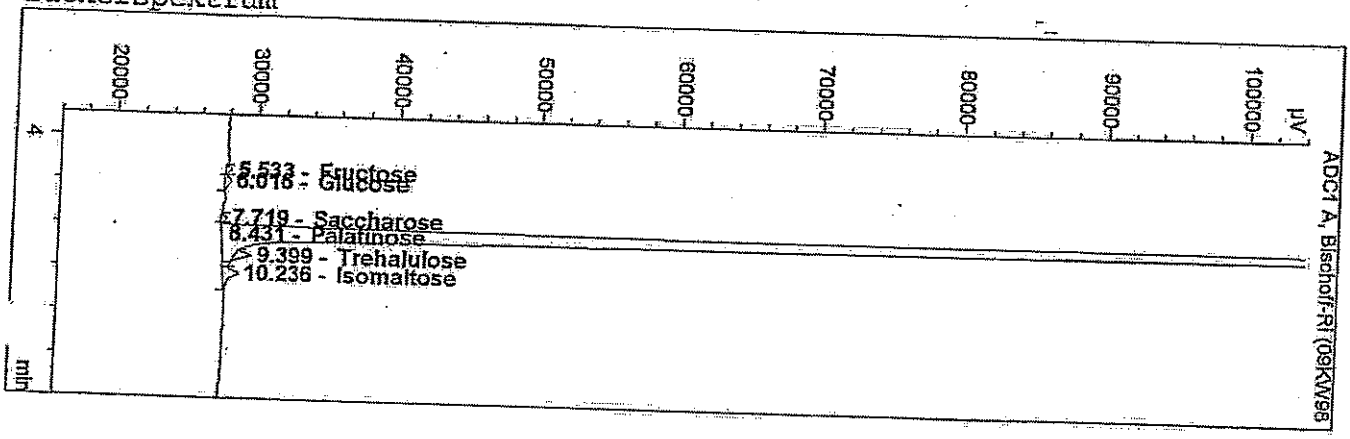
- standard chromatograms (high and low concentration of palatinose)

Data File D:\HPCHEM\ANLAGE6\DATA\09KW98D\SUD_NEU2.D Sample Name: SUD-STD neu

Datum: 26.02.98 RH-1-38

Injection Date	: 27.02.98 08:40:05	Seq. Line :	1
Sample Name	: SUD-STD neu	Vial :	-
Acq. Operator	: Reimund Hermann	Inj :	1
Acq. Method	: D:\HPCHEM\ANLAGE6\METHODS\POSAQUI.M		
Last changed	: 27.02.98 08:35:54 by Reimund Hermann		
Analysis Method	: D:\HPCHEM\ANLAGE6\METHODS\SUD-NH2.M		
Last changed	: 17.03.98 14:47:02 by Reimund Hermann (modified after loading)		

Zuckerspektrum



Normalized Percent Report

Sorted By	:	Retention Time
Calib. Data Modified	:	17.03.98 14:46:59
Multiplier	:	1.0000
Dilution	:	1.0000

Signal 1: ADC1 A, Bischoff-Ri
Uncalibrated Peaks : using compound Palatinose

RetTime [min]	Sig	Type	Area [µV*s]	Amt/Area	Norm %	Grp	Name
5.533	1	MF	5076.41992	2.04869e-6	0.102458		Fructose
6.016	1	FM	9757.76074	1.12731e-6	0.108369		Glucose
7.719	1	MF	5338.02393	2.21056e-6	0.116250		Saccharose
8.431	1	MF R	3.49412e6	2.86115e-6	98.489730		Palatinose
9.399	1	MM T	1.52713e4	4.01406e-6	0.603911		Trehalulose
10.236	1	FM	3.02992e4	1.94065e-6	0.579282		Isomaltose
Totals :					100.000000		

Results obtained with enhanced integrator!

*** End of Report ***

Anlage zur Arbeitsanweisung SZ/A-10.P37

ANNEX 2:

Identification of PALATINOSE using thin layer chromatography.

SÜDZUCKER Standard Operation Procedure Doc.-No. SZ/A-10.P79, Version 10-09-2001

Standard Operation Procedure

SÜDZUCKER

Chapter: 10 Version: 10-09-2001	**Inspection and Testing**	Page 1 of 5 Doc.-No.: SZ/A-10.P79

Identification of Palatinose using thin layer chromatography

1. **Place of Application**

   ZAFES

2. **Responsibility for Performance**

   Laboratory personnel

3. **Supporting Documents**

Doc.-No.	Title	Filing

elaborated:	in conformity with quality system	checked and released	enforced
date: 23.10.01	date: 24.10.01	date: 23.10.01	date: 24.10.01
sign: Martin	sign: Jenel	sign: Pel	

Identification of Palatinose using thin layer chromatography

4. Determination

4.1 Definition

Object of this procedure is the identification of palatinose (isomaltulose) amongst other carbohydrates such as sucrose, glucose, fructose and condensed palatinose. The method is as well suited to distinguish between palatinose and its hydrogenation products glucosyl-sorbitol (GPS) and glucosyl-mannitol (GPM).

4.2 Abstract / Principle

Palatinose can be easily separated and identified by thin layer chromatography using silica gel coated alumina foils and an appropriate solvent as mobile phase. After ascending elution the spots can be detected by dipping the foil into reagent solutions and subsequent heating. Palatinose and the other reference substances can be distinguished by their R_f-values and the colors of the resulting spots.

4.3 Reagents / Material

4.3.1 Equipment / Auxiliary Devices

- TLC-plates: alumina foils, coated with silica gel (e.g.: Kieselgel 60 F_{254}, Merck, 5554)
- Elution chamber
- Filter paper
- Micropipettes
- Chambers for reagent solutions
- Tweezers
- Volumetric flasks, pipettes
- Balance, readable to ± 0.01 g
- Ruler, pencil
- Blast apparatus

Identification of Palatinose using thin layer chromatography

4.3.2 Eluent

Mixture of :

200 ml	ethylacetate
200 ml	pyridine
40 ml	purified water
20 ml	acetic acid (100%)
20 ml	propionic acid

4.3.3 Detecting solutions

Solution A: 0.5 g sodium-metaperiodate in 100 ml of water

Solution B: Cautiously dissolve 10 ml of concentrated sulfuric acid (96%) in 180ml of ethanol using an ice bath and add 2 ml of anisaldehyde and 2 ml of acetic acid (100%).

4.3.4 Reference solutions

Dissolve approx. 0.5 g of each of the following standard substances in 100 ml of purified water:

- Palatinose (Isomaltulose)
- Sucrose
- Glucose
- Fructose

If necessary:

- GPM, GPS (ISOMALT)
- Dipalatinose-dianhydrides

Identification of Palatinose using thin layer chromatography

4.3.5 Sample solution

Dissolve approx. 0.5 g of the solid sample in 100 ml of purified water. Liquid samples are diluted to obtain a concentration of approx. 0.5%.

4.5 Prerequisites for analysis

The elution chamber is filled up to 1 cm with the eluent. A filter paper at the wall of the chamber maintains a saturated environment in the vapor phase.

Cut out TLC-plates of approx. 10 x 10 cm and mark start points.

4.7 Procedure

Apply reference and sample solutions on the start points of the TLC-plate and dry the spots in warm air. Develop the plate in the eluent containing chamber. Mark the eluent front and allow the plate to dry in warm air. Dip the plate for up to 3 sec. in detecting solution A. Dry the plate in hot air. After both sides are completely dried, dip the plate in detecting solution B for up to 3 sec. and dry again in hot air until colored spots become visible. Colors can vary dependent on the applied temperature and are not stable.

4.8 Calculation

Calculate the retardation factor R_f as ratio of the distance traveled by the center of the spot to the distance simultaneously traveled by the mobile phase. The approximate R_f-values, which may slightly vary dependent on the quality of the silica gel plates, are as follows:

Palatinose (Isomaltulose):	0,35
Sucrose:	0,38
Glucose:	0,42
Fructose:	0,50
GPM:	0,17
GPS:	0,14
Dipalatinose-dianhydrides:	0,11

Identification of Palatinose using thin layer chromatography

4.10 Report

R_f-values are given to two decimal places.

5. Appendix

Example chromatogram