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Analysis and Stability of Sucralose in a Milk-Based Confection by a Simple Planar Chromatographic Method

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Sucralose used as high potency sweetener in foods was determined in burfi, a milk-based confection produced in-house. Therefore planar chromatography was employed as a preferred method because of a reagent-free derivatization step. Sucralose was determined on HPTLC amino plates whose amino groups reacted with sucralose to fluorescent zones by just heating the plate after chromatography. Thus derivatization was simultaneously performed for 22 separations per plate, and with ease, over 300 runs can be performed within a day of labor. The within-run precision (*%RSD*) of sucralose determination in milk-based confection was 4.2% ($n = 5$), and the mean recovery 88% ± 4.7% ($n = 6$). LOD via fluorescence measurement was 6 ng/band for standard solutions and 1 mg/kg for the milk-based matrix. According to European legislation, the limits for sucralose addition ranged between 10 and 3000 mg/kg for various foods and thus were fully met with this method. The fluorescence measurement at 366/>400 nm turned out to be slightly more robust and intense than the absorbance measurement at UV 254 nm. The stability of sucralose in milk-based confection was proved under the usual storage conditions at 5, 30, and 45 °C for up to 28 days. Potential hydrolysis products of sucralose caused by various modes of storing the confection were not observed up to 28 days.

KEYWORDS: Sucralose, products of hydrolysis, milk-based confection, amino phases, reagent-free derivatization, food

INTRODUCTION

Escalating prevalence of obesity worldwide and its correlation to other chronic diseases has led to low calorie and sugar free products to move into the main stream food market. The use of high potency sweeteners has emerged as a major way of cutting calorie intake via complete or partial replacement of calorie laden sucrose in numerous food products. Sucralose is a relatively new (although discovered in 1976) no calorie sweetener with a sweetening intensity of 600−650 as compared to sucrose, making it roughly twice as sweet as saccharin and four times as sweet as aspartame. Sucralose shows a taste profile very similar to that of sugar without any aftertaste. The latter is a drawback of many other sweeteners, and this explains the favored application of sucralose in food quality concerns. It is a white to off-white, crystalline, nonhygroscopic, and free-flowing powder. Unlike aspartame, it is stable under heat and over a broad pH range. Because of its exceptional heat stability (mp 130 °C), excellent solubility characteristics, and high compatibility with commonly used food ingredients, it is employed especially in low-calorie products and is known by the trade name Splenda. Since 1991 (first in Canada), it has been approved by the regulatory agencies of more than 60 counties including USFDA for use in numerous food and pharmaceutical products. Recently in January 2005, it has been approved for use in European countries as E955. Chemically, sucralose is 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-(2 → 1)-4-chloro-4-deoxy-α-D-galactopyranoside (**Figure 1**), produced via selective chlorination of sucrose in a five-step process. Thus, as an organochloride, concern over the safety of sucralose is based upon the absence of long-term health studies in humans proving its safety since some organochlorides are known to cause adverse health effects in very small concentrations.

For commercial viability, high potency sweeteners need to be stable during the shelf life of the product in the complex food environment. Few studies on storage stability of sucralose in food products such as beverages and baked goods (*1, 2, 3*) have been reported in literature, but no studies are cited in food systems even similar to milk-based confections. Therefore, this study was carried out to examine the stability of sucralose in a milk-based Indian confection, namely, burfi. Burfi is a popular ethnic milk delicacy, which is typically produced by heat desiccation of milk in an open pan accompanied by continuous stirring until a semisolid mass of about 70% total solids is obtained known as khoa. Then 30% sugar is added to this concentrated mass, that is, khoa on weight basis, which is whipped and molded into the desired shape to obtain burfi. The formulation used for this study was sweetened with sucralose instead of sugar.

Regarding the analysis of sucralose in foods, detection by UV absorption is quite difficult because of the lack of any chromophores, resulting in just a weak UV absorbance (≤200

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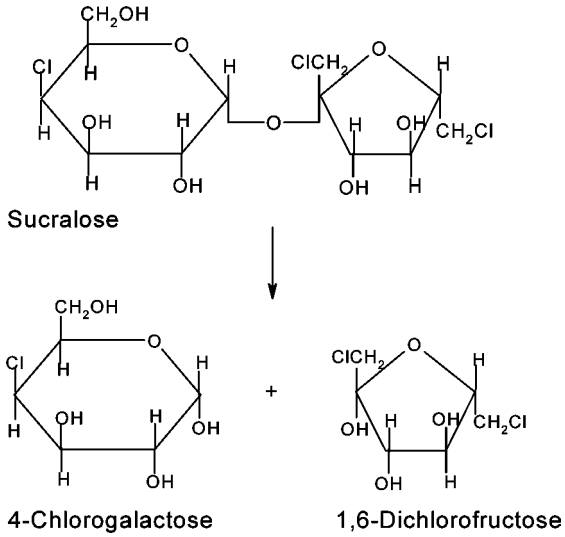

Figure 1. Structure formula of sucralose and its hydrolysis products formed under acidic conditions.

nm). Available RP-HPLC methods employ refractive index detection (*4*−*7*) or UV absorption at 192 (*8*) or 200 nm (*5*). Because of the measurement in the low wavelength range, such methods are less specific and require laborious cleanup to get rid of interfering matrix constituents or high concentrations to be detected. Attempts to derivatize sucralose to a strong UV absorbing derivative by precolumn derivatization with *p*-nitrobenzoyl chloride and detection at 260 nm are very extensive (*9*) and so are LC-MS/MS methods (*10*, *11*). An alternative method to get rid of interfering background is anion-exchange chromatography with pulsed amperometric detection (*6*, *12*). Also, capillary zone electrophoresis (CE) has been used with indirect ultraviolet absorption at 238 nm in a 3,5-dinitrobenzoic acid buffer (*13*, *14*) but shows shifts in migration times and interferences with fructose or other sugars if these are present in higher amounts. A new planar chromatographic method for sucralose (*15*) employed the reagent-free derivatization on amino phases by just heating the plate after chromatography, an elegant way of derivatization well known for the analysis of sugars (*16*). The mechanism is supposed to be a Maillard reaction forming high molecular mass derivatives of fluorescent nature (*17*). A benchmarking of all this methods showed planar chromatography to be advantageous as a simple but reliable method showing better repeatability and lower LOD as CE. Moreover, the derivatization step to fluorescent zones can rapidly and simultaneously be performed for all substance zones on a plate. Planar chromatography is very tolerant to less sample preparation and besides cost-effectiveness another reason for the decision to employ HPTLC for this study.

MATERIALS AND METHODS

Materials. Milk was obtained from Institute of Animal Foodstuff Technology at University of Hohenheim. Maltodextrin and sorbitol were provided by Crestar (Krefeld, Germany) and Nestle (Biessenhofen, Germany), respectively. Sucralose (batch No. D0304B36MA) was supplied by Tate & Lyle Specialty Sweeteners (Reading, England). All reagents used were of analytical grade unless otherwise described. Acetonirile and methanol (both technical grade) were in-house distilled before use. Ultrapure water was produced by Synergy System (Millipore GmbH, Schwalbach, Germany). For preparation of Carrez I solution, potassium hexacynoferrate (II) trihydrate ($K_4[Fe(CN)_6]\cdot 3\ H_2O$) was dissolved in water (3.6 g in 100 mL). Carrez II solution was prepared by dissolving zink sulfate hepta hydrate ($ZnSO_4\cdot 7\ H_2O$,) in water (7.2 g in 100 mL). HPTLC plates NH_2 F_{254} (20 × 10 cm, Art. no. 1.13192.0001) were supplied by Merck (Darmstadt, Germany). As solutions for fluorescence enhancement, *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide was dissolved in methanol (5 g in 100 mL) and paraffin in *n*-hexane (1:1, v/v).

Processing of Burfi (Burfi Making). Burfi samples were prepared in the Dairy for Research and Training, Institute of Animal Foodstuff Technology at University of Hohenheim, employing the process and formulation standardized at National Dairy Research Institute, India. The detailed formulation which will be published elsewhere contains an optimized combination of bulking agents (maltodextrin and sorbitol) and sucralose to replace sugar. The product was sweetened by adding 0.0375% (w/w) sucralose to khoa.

Storage of Samples. Burfi samples packed in polyethylene pouches were stored at three temperatures, 45 ± 1 °C, 30 ± 1 °C, and 5 ± 1 °C, for 1, 2, and 4 weeks to comprise the usual storage conditions of the product and some accelerated conditions. Samples were withdrawn according to a predefined schedule and stored at −20 °C until analysis was performed.

Moisture Content. The method as described in IS 2785-1964a (*18*) for determination of moisture in cheese was used for burfi with slight modifications. About 10 g of previously washed and dried sand was weighed into an aluminum dish and allowed to dry further in an oven maintained at 100 ± 5 °C along with a glass rod. After cooling in a desiccator, a 2 g burfi sample was transferred into the dish, and 5 mL of distilled water was added to it. The contents were mixed thoroughly with the help of a glass rod. The moisture dishes were then transferred on a thermostatically controlled water bath at 100 ± 1 °C for 30 min and later transferred to an oven maintained at 100 ± 5°C. Drying was continued until the solid mass of the dishes turned to be light brown and the difference between two successive weightings, each after cooling in a desiccator, was not more than 1 mg. The result was expressed on the basis of 100 g of burfi as follows:

$$\text{moisture (\% by mass)} = \{(C - A)/W\} \times 100$$

where C is the weight (g) of the dish (including sand and glass rod) with sample before drying, W is the weight (g) of burfi sample taken, and A is the weight (g) of the dish (including sand and glass rod) with sample after drying.

Extraction of Sample. A representative aliquot of the sample (5 g) was transferred to a 50 mL volumetric flask and suspended in 20 mL water by shaking. Methanol (20 mL) was added to precipitate the proteins. Extraction of sucralose was carried out in an ultrasonication bath for 15 min. Carrez I and Carrez II solutions (2 mL each) were added successively to further precipitate soluble proteins. Water was added to obtain the final volume of 50 mL. After centrifugation at 4000 rpm for 10 min, aliquots were filtered through a 4.5 μm membrane filter before application onto the stationary phase. The same sample was extracted twice and analyzed as a 2-fold determination.

Standard Solution. As stock solution (1.5 mg/mL), sucralose was dissolved in methanol. For application, the stock solution was diluted 1:100 with methanol (standard solution).

Generating Products of Hydrolysis. A 20 mL aqueous solution of sucralose (2.5 mg/mL) was treated with 1 mL hydrochloric acid (37%) and heated up to 80 °C for 15 min in an oven. For application, the stock solution was diluted 1:200 with methanol.

Application. For quantitative determination, the solutions were sprayed with the Automatic TLC Sampler 4 (ATS4, CAMAG, Muttenz Switzerland) as 5 mm bands allowing 22 tracks to be applied on one HPTLC plate of 20 × 10 cm (distance from lower edge 8 mm, distance from the left side 15 mm, and distance between bands 7.8 mm). For calibration, the standard solutions were applied in volumes of 2 to 10 μL, that is, 30−150 ng per substance zone absolutely on the HPTLC plate. From sample solutions, 4 μL each were sprayed-on as the starting zone. For determination of the within-run precision, the burfi sample was applied 5-fold. For routine analysis, each sample and each standard concentration was applied twice on the plate.

Chromatography. Chromatography was performed on HPTLC plates NH_2 F_{254} with acetonitrile and water (4:1, v/v) in a Horizontal

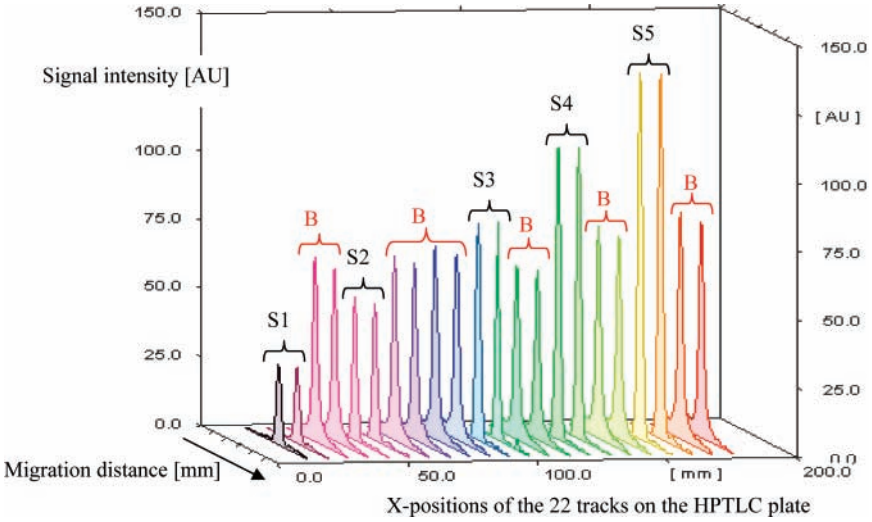

Figure 2. Substance windows of sucralose standards and samples of all 22 tracks on one plate (10 cm × 20 cm); sucralose of standard tracks ranged from 30–150 ng/band (S1–S5) and different burfi samples; florescence scan at 366/>400 nm.

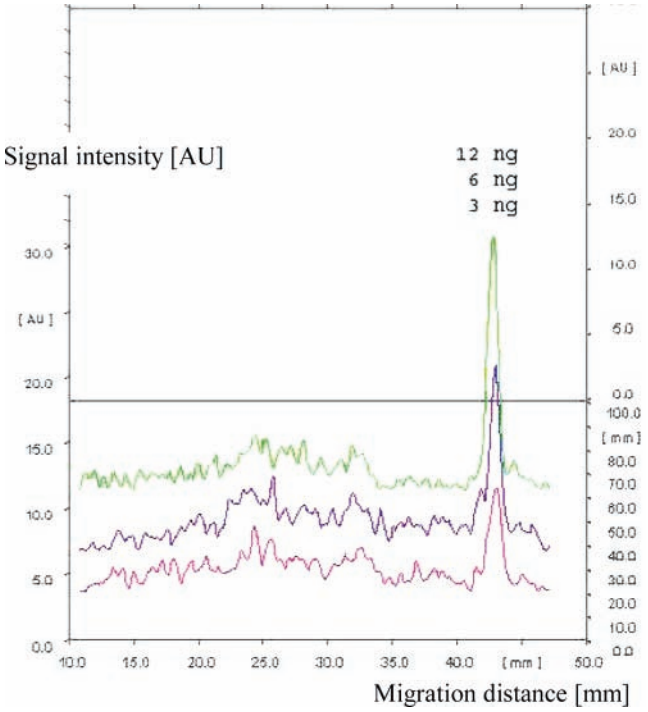

Figure 3. Three-dimensional overlay of three standard tracks obtained by fluorescence measurement at 366/>400 nm; LOD (S/N 3) was determined to be 6 ng/band for standard solutions without any use of fluorescence intensifying agents.

Developing Chamber with tank configuration (HDC, CAMAG, Muttenz Switzerland) up to a migration distance of 70 mm (about 15 min migration time). For thermal in situ derivatization of sucralose zones, the developed plate was dried under a hair dryer for at least 5 min and then heated on the TLC plate heater III (CAMAG, Muttenz Switzerland) at 190 °C for 20 min.

Evaluation. Densitometric evaluation was performed by TLC Scanner 3 (CAMAG, Muttenz, Switzerland) via fluorescence measurement at UV 366/>400 nm (slit size 3 mm × 0.45 mm, scanning speed 20 mm/s). Calibration range was 30−150 ng of sucralose absolutely on the HPTLC plate. Plate images were documented by DigiStore Documentation System (CAMAG, Muttenz, Switzerland) consisting of illuminator Reprostar 3 with digital camera Powershot G2 (Canon Inc., Tokyo, Japan). Data obtained was processed with winCATS software, version 1.26 (CAMAG, Muttenz, Switzerland).

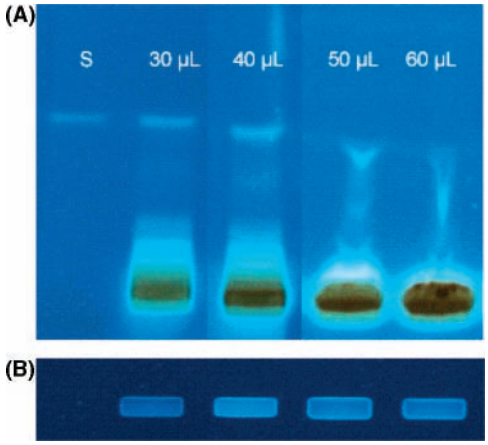

Figure 4. Application as area (10 × 5 mm², **A**) enables application of higher sample volumes (up to 40 µL) to obtain a LOD of 1 mg/L in the milk-based burfi sample; higher volumes (≥50 µL) had an adverse affect on chromatography (**B**); track 1, sucralose standard solution (S), track 2–5 (collage from different plates), different volumes of burfi samples applied (30–60 µL). (This LOD was highly sufficient; thus, an increased area of 10 × 5 mm² in combination with volumes higher than 40 µL has not been tested to further improve the LOD in matrix.)

RESULTS AND DISCUSSION

For high-throughput analysis, it was of interest to get as much samples on one HPTLC plate as possible. Thus, for analysis of sucralose, 22 tracks were arranged on one HPTLC plate (20 × 10 cm), meaning 22 separations were performed simultaneously within 15 min (**Figure 2**). For application, a minimum band length of 5 mm was chosen to enable homogeneous derivatization of sucralose with sufficient amino groups of the stationary phase and an optimal detectability. The 5 mm-bands were the best compromise between sample load and good precision of the results in the calibration range shown.

Chromatography was performed on amino phases with a mixture of acetonitrile and water up to a migration distance of 70 mm. In contrast to Spangenberg et al. (*16*), a higher migration distance of 70 mm, instead of 50 mm, improved the precision

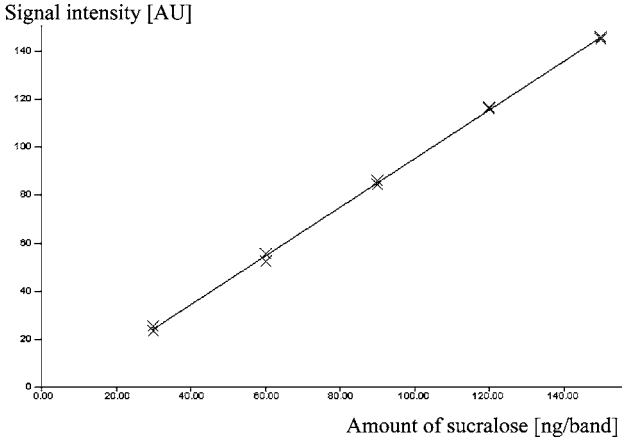

Figure 5. Linear calibration ($y = 1.011 \times -6.117$) via peak height by fluorescence measurement at 366/>400 nm in the range between 30–150 ng/band with a relative standard deviation of the calibration function (sdv) of ± 1.42% and a correlation coefficient of 0.9997.

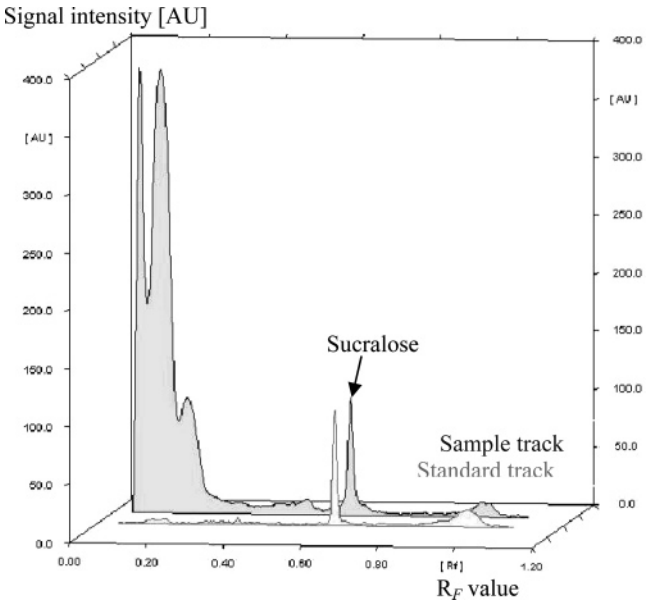

Figure 6. Overlay of a standard (sucralose at hR_F 62, lower curve) and sample track (upper curve) showing that the milk-based matrix is clearly fixed in the lower hR_F range. Fluorescence scan at 366/>400 nm after chromatography on amino phases with a mixture of acetonitrile and water up to a migration distance of 70 mm followed by heating at 190 °C for 20 min.

for the given milk-based matrix and additionally allowed separation and monitoring of the hydrolysis products. After chromatography, the HPTLC plate was heated on the TLC plate heater at 190 °C for 20 min. Performance of the derivatization at lower temperature and shorter period led to less intense fluorescent signals.

To the best of our knowledge, limit of detection (LOD) and limit of quantification (LOQ) have not been established so far for sucralose in the fluorescence measurement mode, although it is the most reasonable and superior detection mode when compared to absorbance measurement. It is superior because sucralose migrates with the β-front. In the absorbance measurement mode, interferences caused by UV-absorbent impurities, which could be fixed and visible in such a β-front, could make quantitative evaluation impossible. In other words, plate prewashing and working with very pure solvents are prerequisites for such a β-front evaluation. However, for fluorescence

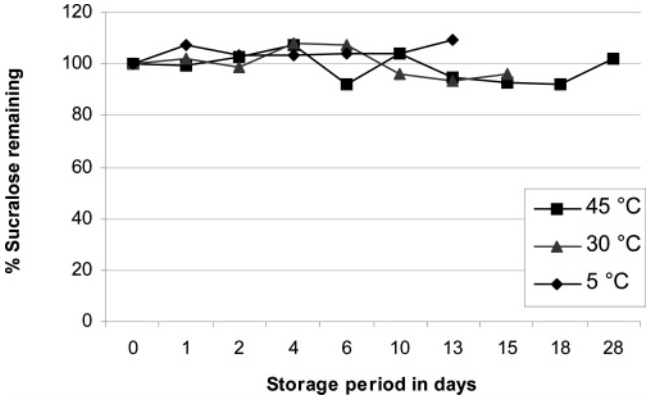

Figure 7. Percent sucralose concentration remaining during the storage period of burfi at three different temperatures; respective data are shown in **Table 1**.

Table 1. Sucralose Concentration Remaining (%) during the Storage Period of burfi at Three Different Temperatures

	sucralose concentration remaining (%) at storage temperature		
storage period (days)	5 °C	30 °C	45 °C
0	100.0	100.0	100.0
1	107.2	101.9	99.3
2	103.7	98.8	103.0
4	103.2	107.9	107.2
6	104.1	107.1	92.0
10	103.7	96.0	104.1
13	109.3	93.2	94.7
15		96.3	93.0
18			92.4
28			102.4
mean ± RSD	104.5 ± 3.0 (n = 7)	100.2 ± 5.2 (n = 8)	98.8 ± 5.5 (n = 10)

Table 2. Moisture Content (%) of the First and Last Sample Stored at Different Temperatures

	moisture (% by mass)	
storage temperature (°C)	before storage (fresh sample)	at the end of the storage period (d)
5	33.6	33.5 (13)
30	33.6	31.7 (15)
45	33.6	28.3 (28)

measurements, on the basis of the selective derivatization of sucralose, this interference was not observed. For absorbance measurements, LOD has been reported to be 1 mg/L in the matrix and 5 ng/zone in the HPTLC plate (*16*). For fluorescence measurements, similar limits were established. The LOD (signal-to-noise (S/N) ratio of 3) was 6 ng/band for standard solutions (**Figure 3**) and 1 mg/L for the milk-based matrix when sample volumes of 40 μL were applied. Application of such 40 μL-burfi samples required area application to avoid a chromatographic barrier caused by the heavy milk matrix at the starting zone. Up to a 40 μL burfi sample could be applied as a 10 × 3 mm^2 area without any adverse effect on chromatography (**Figure 4**) leading to an LOD of 1 mg/L. According to European legislation, the limits for sucralose addition ranged between 10 and 3000 mg/kg for various foods and thus were fully met with this method. Hence, in our opinion this LOD was sufficient, and an increased area, for example, of 10 × 5 mm^2, in combination with volumes higher than 40 μL have not been tested to further reduce and improve the LOD in matrix. For

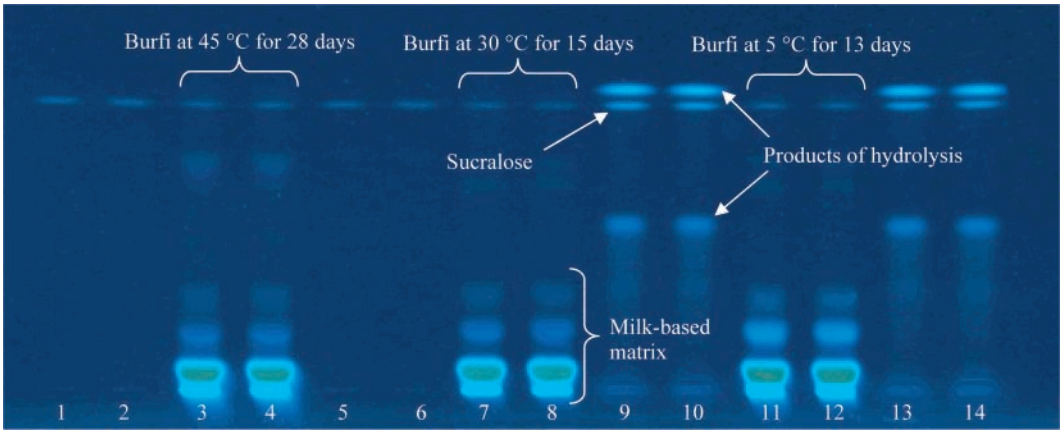

Figure 8. Plate image depicting the absence of products of hydrolysis in burfi samples stored until the end of each temperature set up; documentation via illumination (reflectance) at UV 366/>400 nm. Tracks 1, 2, 5, and 6, sucralose standard, which is blue fluorescent at hR_F 62; tracks 9, 10, 13, and 14, sucralose standard solution subjected to hydrolysis; tracks 3, 4, 7, 8, 11, and 12, burfi samples stored at 45 °C for 28 days, 30 °C for 15 days, and 5 °C for 13 days (2-fold extraction each), respectively.

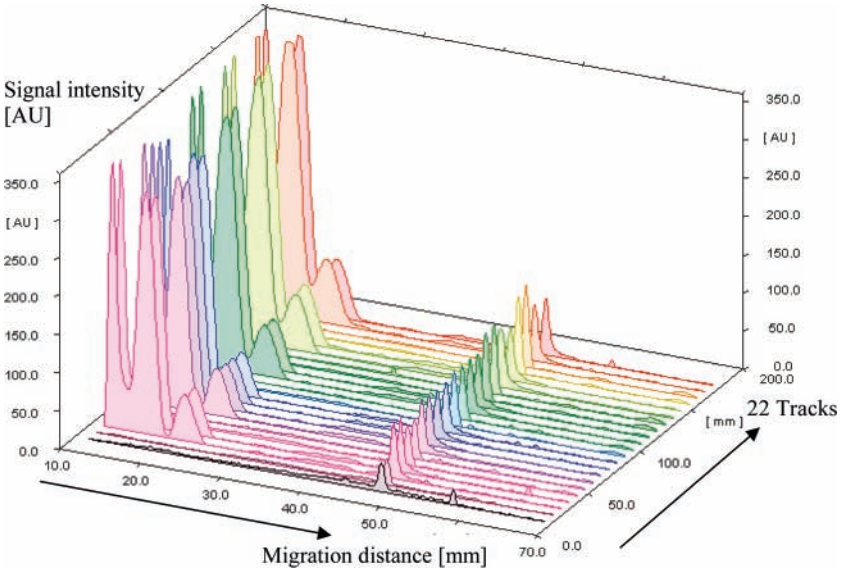

Figure 9. Parallel chromatography (22 runs take 15 min), minimized sample preparation (matrix is left at the starting zone), and simultaneous derivatization of 22 runs (just heating of the plate) contribute to the high-thoughput capacity of planar chromatography. Hence, over 300 runs can be performed with ease within a day of labor (by one person at one cost-effective HPTLC work station).

routine burfi analysis, the application of 4 μL-sample volumes was highly sufficient.

Quantification of sucralose was very good. A representative calibration in the range between 30−150 ng with a relative standard deviation of the calibration function ($y = 1.011 \times -6.117$) of ± 1.42% and a correlation coefficient of 0.9997 is given in **Figure 5**. Evaluation via peak height was slightly better than that via peak area. The within-run precision (*%RSD*) of sucralose determination in the milk-based confection was 4.2% ($n = 5$), and the mean recovery rate was found to be 87.5% ± 4.7% ($n = 6$).

The mobile phase system was very well suited to separate sucralose (hR_F62) from the fluorescent milk-based matrix, that is, mainly sugars such as lactose, galactose, or glucose. **Figure 6** shows the separation for a sample and standard track. About 40 relevant substances, which can be derivatized on amino plates to fluorescent substances as well, were checked regarding coelution, and no interference was observed. Carbohydrates as well as amino acids showed hR_F values in the range of 0−20, organic acids in the hR_F range of 0 to 10, and other sweeteners over the whole migration distance (hR_F 25 to 70). Polyols were not detected.

For commercial viability, it was of interest that sweeteners are stable during the shelf life of the product. So far, no studies on storage stability of sucralose in food systems similar to milk-based confections have been performed. Thus, to the best of our knowledge, this is the first time hydrolysis products of sucralose were monitored in milk-based products, which were wrapped in polyethylene pouches and stored at three temperatures, 45, 30, and 5 °C, over a period of up to 28 days to comprise the usual storage conditions of the product. For analysis, each sample withdrawn in defined time intervals was extracted in duplicates, and each extract was applied twice on the HPTLC plate. At low temperature, fewer samples were taken for analysis, expecting less or no degradation than the samples at higher temperatures. HPTLC analysis of burfi samples revealed that no change in sucralose concentration was observed over the time investigated, even at elevated temperatures (**Table 1**). According to the *t*-test (99%, *t*-table value 2.95; calculated *t*-value 2.48) the three mean values were not significantly

different and thus comparable, meaning that until the end of the storage period similar sucralose concentrations were obtained at the three storage temperatures. At higher temperatures, uneven moisture loss can be responsible for increased fluctuations in the results (**Figure 7**). The moisture content (dry matter) was determined at the beginning and at the end of each temperature setup (**Table 2**). The sample stored at 5 °C showed no moisture loss, whereas the one stored at 45 °C showed a minor reduction in moisture content and thus dry matter increase.

Regarding this data, it could be concluded that no major change in sweetness level or major loss in sensory quality due to sucralose degradation could be expected for the complete shelf life of the burfi product. However in terms of safety, "because sucralose may hydrolyze in some food products ... the resulting hydrolysis products may also be ingested by the consumer", as noted by the FDA, our study was also focused on products of hydrolysis. Hence, for further confirmation of sucralose stability in burfi, the last withdrawn samples from each storage temperatures were checked for the presence of any of the two probable hydrolysis products. These samples were developed along with the sucralose hydrolysis products generated in our laboratory because at that time the products of hydrolysis (**Figure 1**), that is, 1,6-dichlorofructose (1,6-DCF) and 4-chlorogalactose (4-CG), were not commercially available. Referring to the given detection sensitivity, **Figure 8** clearly shows the absence of any hydrolysis products in burfi samples stored until the end of each temperature setup. No peak was observed at the same R_F values as those of the generated products of hydrolysis in all samples tested, confirming that sucralose was stable under the selected storage conditions. For the first time, products of hydrolysis were monitored. It was proven that no interference was caused by potential products of hydrolysis in the separation system given.

For improvement of the heat-induced fluorescence signal, several agents used for fluorescence stabilization and intensification were investigated. Fluorescence enhancement by dipping the plate in a methanolic solution of *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide generated only a very slight intensification but not an enhancement by a factor of 2 (*16*). Further solutions for enhancement tested were triethylamine/dichloromethane (1:9, v/v), Triton X100/methanol (1:4, v/v), PEG 2000/methanol (1:1, v/v), paraffin/toluene (1:4, v/v), paraffin/*n*-hexane (1:2, v/v), and paraffin/*n*-hexane (1:1, v/v). They all generated only intensifications of less than a factor of 2 (plus 42 to 67%) and thus were not of major importance. Fluorescence signal intensity of sucralose was proved to be stable for 60 h if the plate was stored in the dark. A longer period of time was not studied.

Conclusions. Planar chromatography was well suited for the investigation of the stability of sucralose in a milk-based confection. The reagent-free derivatization to fluorescent zones and the subsequent fluorescence measurement was found to be highly selective and slightly more robust than the absorbance measurement. The within-run precision (*RSD* 4.2%) of sucralose determination in the matrix and the mean recovery rate (88%) demonstrate the suitability of this simple method. LOD was 6 ng/band for standard solutions and 1 mg/kg for milk-based matrix (area application). Thus, the required limits according to European legislation (10−3000 mg/kg) were fully met. Up to 28 days and in different storage modes of the confection (at 5, 30, and 45 °C), a significant change in the sucralose concentration in burfi was not observed. Additionally, monitoring of the two products of hydrolysis was possible, and none was detected in the maximal stored samples of each temperature setup. The sensory evaluation carried out by a trained sensory panel at the National Dairy Research Institute (NDRI) in Karnal, India, judged the dietetic burfi to have the sweetness intensity very close to that of the optimum (score 5) up to 18 and 6 days when stored at 5 and 30 °C, respectively (*19*). The data showed that dietetic burfi sweetened with sucralose maintained the same sweetness intensity and quality throughout the normal shelf life span, and this led to the recommendation of sucralose as a sweetener for burfi.

The analysis of 22 samples required 50 min of inclusive application (10 min), parallel chromatography (15 min), simultaneous derivatization (just heating for 20 min), and evaluation (5 min). This means an analysis takes 2.3 min, which demonstrates the high-thoughput capacity of planar chromatography. Sample preparation is not included in this calculation; however, as demonstrated in **Figure 9**, the sample preparation can be minimized as the HPTLC plate is used only once. Above all, the through-put can even be doubled because during application and development of one plate, another plate can be derivatized and evaluated, an advantage of the offline work station and its staggered system. Hence, with ease, over 300 runs can be performed within a day of labor (by one person at one HPTLC work station) with cost-effective instrumentation. On the basis of the positive experience of sucralose quantification with planar chromatography, an interlaboratory test was performed in the spring of 2007 organized by the Institute of Reference Materials (IRM) in Gheel, Belgium.

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