

RESEARCH ARTICLE

Investigation of sucralose degradation in high-temperature treatment of low-calorie *basundi*

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Abstract: High-temperature processing (HTP) is an effective method for extending the shelf life of foods, ensuring that they remain preserved regardless of their water activity levels. *Basundi* is an Indian heat-desiccated traditional milk product. Because of its highly sugar content low calorie *basundi* was developed using sucralose as high intensity sweetener. This investigation aimed to check the hydrolysis of sucralose at high-temperature processing (115±1 °C for 5 min) of low-calorie *basundi* stored at 30±1 °C in a polypropylene (PP) container. The milk was concentrated to 20% then simmered at 90±1 °C for 17 min after the addition of 0.162 g sucralose. The charred spots obtained on developed aluminium-backed HPTLC silica gel 60 F254 (kiesel gel 60 F254) plates for the sample isolated from low-calorie *basundi* showed intensity values of 239.2 for a 10 µL sample on the 15th and 30th days (based on grey scale). The software Image J 1.52a measured the intensity of the scanned images of the charred spots developed on HPTLC plates. High-performance thin-layer chromatography (HPTLC) analysis showed the same concentration of sucralose (1425 µg in a 10 g product) on the 15th and 30th days. Thus, it confirmed the stability of sucralose at high-temperature processing (HTP), 90±1 °C for 17 min, and storage for 30 days. The findings hold great potential for manufacturing and extending the shelf life of low-calorie *basundi* and similar room-storable milk products.

Keywords: Milk, Low-calorie *basundi*, Sucralose, High-temperature processing (HTP), High-performance thin-layer chromatography (HTPLC), Shelf-life

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Introduction

Controlling water activity (a_w) is a proven approach to preserve food. The foods with low water activity (a_w) are considered low-risk for the proliferation of non-pathogenic bacteria; however, there have been reports of illness outbreaks associated with these foods (Ijabadeniyi and Pillay 2017; Wason et al. 2021). It is not recommended to maintain low a_w in every milk product and prevent spoilage as it increases shelf-life at the cost of food quality (Putnik et al. 2020). Therefore, other methods like retorting, ultra-high temperature (UHT), and high-temperature treatment are highly recommended for the long shelf-life of milk-based products (Joyce et al. 2018; Rasane et al. 2020). The sweetened condensed milk, evaporated milk, and the *basundi* have a_w values between 0.77 and 0.88, 0.98 and 0.99 (Chawla 2018), and 0.97 and 0.98 (Patel and Upadhyay 2003), respectively.

The current investigation aimed to explore the effect of high-temperature processing (HTP) on sucralose stability when the low-calorie *basundi* containing it stored at 30±1 °C. It is essential because the popularity and consumption of the *basundi* have spread internationally with demand in the USA, West Indies, Africa, Gulf Region, SAARC neighbours, Singapore, Philippines, Thailand, Japan, and China (Kaur 2014). The product was manufactured by replacing sucrose with 65 ppm sucralose and 5% a mixture of bulking agents in an equal ratio (maltodextrin and D-sorbitol, 1:1) and inulin (1.5%) as a fat replacer.

Sucralose is a no-calorie sweetener. It is 600 times sweeter than sucrose (Bannach et al. 2009; Chattopadhyay et al. 2014). As a chlorinated derivative of sucrose, it replaces three hydroxyl groups with chlorine atoms at the 4', 1', and 6' positions to form 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside (Jenner and Smithson 1989; Schiffman and Rother 2013). It is most stable in an acidic pH range of 5–6 and is highly soluble in water (28.3 g for 100 mL at 20 °C) (Jenner and Smithson 1989; Magnusan et al. 2017). At high temperatures due to low and high pH sucralose hydrolysis to 4-chlorogalactose; 1,6-dichlorofructose, uronic acid, and 3,6-anhydro-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside, respectively (De Oliveira 2015; George 2011).

Sucralose cannot be estimated using the HPLC separation technique with a UV detector because it lacks chromophores (Chen et al. 2025). Therefore, the HPTLC technique was applied for the analysis of sucralose in food matrices such as yogurt, jam, powders, starch-based puddings, and soft drinks (Spangenberg et al. 2003; Stroka et al. 2009; Vidushi and Meenakshi 2017).

Sucralose's increasing popularity has led researchers to examine its physicochemical properties under different processing conditions. Despite its generally recognized as safe (GRAS) status, caution is advised when using sucralose as a sweetener in cooked and baked foods containing glycerol or lipids due to the potential formation of hazardous chloropropanols (Rodero et al. 2009; Rahn and Yaylayan 2010). Bannach et al. (2009) studied the instability of sucralose using differential thermal analysis (DTA) and reported pyrolysis occurred in three steps i.e., 119–137 °C, 160–370 °C, and 370–500 °C when dissolved in water. De Oliveira et al. (2015) reported the breakdown of sucralose crystals with the generation of polychlorinated aromatic hydrocarbons (CAHs) at the temperature of 98 °C (simmering temperature of milk tea, and coffee). Also, the generation of CO₂ and hydrogen chloride, the melting of the sucralose crystal followed by its decomposition, and CAHs at 125 °C. Hellwig (2024) reported significant instability and discoloration of sucralose after being heated at 85-90 °C for 1 hour. High-Performance Liquid Chromatography-Time-of-Flight Mass Spectrometry (HPLC-TOF-MS) identified a chlorinated furan-3-one, 4-chlorogalactosyl residue and a 1,6-dichlorofructosyl residue. The researcher agreed to the findings of the German Federal Institute for Risk Assessment, which indicated a persistent gap in knowledge regarding the identity of the end products resulting from cooking reactions. But, the studies conducted by Barndt and Jackson (1990) and Eisenreich et al. (2020) supported the stability of sucralose in baked foods. Berry et al. (2016); Magnuson et al. (2017); Rao and Pagote (2018) and Gujral et al. (2021) also supported the stability of sucralose at the high-temperature treatment of cooked and baked foods.

Although many researches exist on the stability of sucralose in food products, no research has been reported to date to investigate the effect of HTP on the stability of sucralose in low-calorie *basundi* stored at high temperatures. Therefore, there is an urge to investigate the sucralose stability in low-calorie *basundi* during HTP and its storage at high temperatures.

This knowledge would be valuable for the HTP, manufacture, and shelf-life enhancement of other low-calorie milk-based products like milk-based puddings/desserts (*burfi*, *kheer*, *payasam*), and heat-desiccated products (*kulfi*, *rabri*, and *khoa* based sweets) storable at room temperature. In this study, an attempt was made to investigate the degradation of sucralose in HTP (115±1°C for 5 min) of low-calorie *basundi* packaged in a

sterilisable polypropylene (PP) container and stored at 30±1 °C using an HPTLC technique.

Materials and methods

Ingredients

Raw buffalo milk (fat, 9.0-9.3%; solids-not-fat (SNF), 10.13-10.98%; acidity, 0.13-0.14% lactic acid) was procured from the experimental dairy plant of ICAR-NDRI. Food grade sucrose was purchased from Dhampur Sugar Mills Ltd., Mansurpur, Muzaffar Nagar, Uttar Pradesh, India. Sucralose (off-white powder; specific rotation, +87.29 °; melting point, 130 °C; solution (10%) at 25 °C had a pH of 6.30), maltodextrin (a white to off-white color powder; dextrose equivalent (DE), 14.33; solubility in water at 25 °C, 100 mg mL⁻¹), and D-sorbitol (white crystals; specific rotation, 108.60 °; solubility in water at 25 °C, 1 g mL⁻¹), were purchased from M/s Hi-Media Laboratories Pvt. Ltd. supplied by Nu-Scientific Biotechnologies, Karnal, India. Inulin variant Orafiti® HPX (a degree of polymerization (DP), more than 23; sweetness level, 0%) suitable for fat replacement and heat-treatment above 105 °C, was purchased from SFA Food and Pharma Ingredients Pvt. Ltd., Wagle Industrial Estate, Thane (W), Maharashtra, India. All the ingredients used were of HPLC grade.

Chemicals

The aluminium-backed HPTLC silica gel 60 F₂₅₄ (Kiesel gel 60 F₂₅₄) plates (20×20 cm²), acetonitrile, dichloromethane, and methanol were purchased from M/s Hi-Media Laboratories Pvt. Ltd. supplied by Nu-Scientific Biotechnologies. The HPLC grade water was purchased from Qualigens, India. Carrez solutions no. 1 and no. 2 were prepared by dissolving 3.6 g of potassium ferrocyanide and 7.2 g of zinc sulfate each, in 100 mL HPLC grade water (George et al. 2010).

Type of equipment

The centrifugal cream separator (Kamdhenu, Benny, India), shallow bottom pan (stainless steel, SS 304) and the milk-scrapper (SS 304), handheld refractometer (28–62 °Brix), ultra-sonifier (SONICS, Vibra cell, model VC×750, Newton, CT, USA), biological oxygen demand (BOD) incubator (Narang Scientific Works Pvt. Ltd., India), electronic balance (Mettler AT-200, Switzerland), hot air oven (Akashdeep, Delhi), filter paper (Whatman no. 1, England), loop injector (10 µL) and a software Image J 1.52a provided by the National Institute of Health (NIH), USA, publicly free to access (<https://imagej.nih.gov/ij/download.html>) were used in the study.

Instrumental colour analysis

Color determination was performed using a Hunter Lab Colour Flex® reflectance meter provided by Hunter Associates Laboratory Inc. in Reston, Virginia, U.S.A. The tri-stimulus values

(L^* , a^* , b^*) were measured under standard illumination conditions of D65 (6500 K, daylight) and at a viewing angle of 10° , as recommended by International Commission on Illumination (CIE) standards 1971 (Cheng et al. 2018).

Packaging material

The sterilisable PP container having a capacity of 100 mL was used for HTP of low-calorie *basundi* in an autoclave cum sterilizer (capacity: 50 L; brand: TENSO). The container had excellent heat and chemical resistance properties with density ($0.895\text{--}0.92\text{ g cm}^3$), melting point ($200\text{--}290\text{ }^\circ\text{C}$), and was procured from Tarsons products Pvt. Ltd, supplied by Nu-Scientific Biotechnologies, Karnal, Haryana, India.

Manufacture of high-temperature processed low-calorie *basundi*

The optimization of ingredients for the manufacture of low-calorie *basundi* was performed by Singh et al. 2022. For its preparation, the buffalo milk was standardized to 0.5% fat and 9.0% SNF (w/w, wet basis) using Pearson Square method (Bird, 1993). The standardized milk was then boiled at (approximately) $100.5\text{ }^\circ\text{C}$ in a shallow *karahi* with continuous string cum scrapping till concentration reached about 20% total milk solids (TMS). The temperature of the milk concentrate was decreased to $90\pm 1\text{ }^\circ\text{C}$ that took $57\pm 1\text{ s}$, by throttling the fuel supply valve to the heater. At this stage, a mixture of maltodextrin and D-sorbitol in an equal share (5% of milk, w/w), sucralose (65 ppm of milk, w/w), and inulin (1.5% of milk, w/w) were added to the milk concentrate in the form of a slurry having 25% TS (w/w). The mixture was further concentrated at $90\pm 1\text{ }^\circ\text{C}$ for 17 min to achieve 36% TS (w/w) called low-calorie *basundi* (Singh et al. 2022). A portable handheld refractometer was used to measure total soluble solids (TSS) or $^\circ\text{Brix}$, and this TSS value was put in the equation (1) mentioned below as suggested by Moore et al. (2009) to know TS%. A pilot sterilization test was conducted to check the heat stability of the product (Table 1). The stabilizer selected disodium phosphate added at 0.012% of the low-calorie *basundi* before the HTP to increase the heat stability of the low-calorie *basundi*. The product was then packaged in sterilisable PP containers sealed with PP lids. The sealed containers were then processed at $115\pm 1\text{ }^\circ\text{C}$ for 5 min and stored at $30\pm 1\text{ }^\circ\text{C}$ in the incubator for further analysis. The heat treatment mode was in-container sterilization performed in an autoclave. The calibration process took $17\pm 1\text{ min}$ to bring the autoclave temperature to $115\pm 1\text{ }^\circ\text{C}$. After HTP of low-calorie *basundi*, the steam pressure was released which took 2 min (approximately). The product was cooled at room temperature and then stored in the incubator. The flow chart depicting the manufacturing process of low-calorie *basundi* for a batch of 2.5 kg of milk is shown in Fig. 1.

$$\text{TS\%} = 0.9984 (\% \text{ Brix}) + 2.077 \quad (1)$$

Preparation of a standard curve

The standard curve was a plot at the concentration of sucralose to the intensity of charred spots. The concentration formed the x-axis and the intensity the y-axis. The eight different concentrations were plotted viz., 0.5, 0.65, 1.0, 3.0, 5.0, 7.0, 9.0, and $11.0\text{ }\mu\text{g } 10\text{ }\mu\text{L}^{-1}$ in increasing order of concentration from left to right on the plate (Fig. 2). For this different amount of HPLC grade sucralose viz., 0.0125, 0.01625, 0.025, 0.075, 0.125, 0.175,

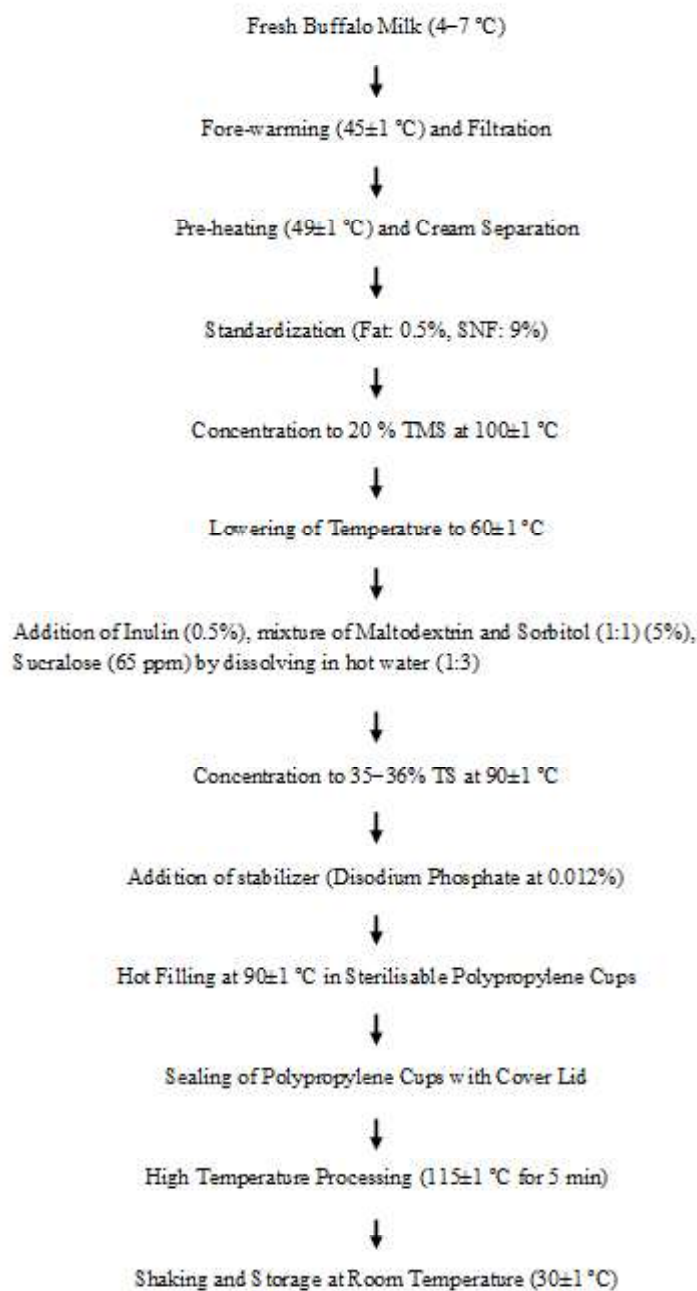


Fig. 1. Process flow diagram of high-temperature processed low-calorie *Basundi*

0.225 and 0.275 g, was mixed thoroughly, each in 250 mL HPLC grade water at 25 °C. HPTLC analysis of standard sucralose solution in combination with software Image J 1.52a correlated the intensity to concentrations. The method for running standard sucralose was the same as for a low-calorie *basundi*. The intensity of samples was measured using the above-mentioned software. The regression equation and correlation coefficient (R^2) obtained for the standard curve were $(-3.888x + 244.84)$ and 0.99, respectively. The plot gave a linear inverse relationship between the concentration ($\mu\text{g } 10 \mu\text{L}^{-1}$) and intensity (based on grayscale), as tabulated in Table 2 and shown in Fig. 2.

Estimation of sucralose in low-calorie basundi

The estimation of sucralose was carried out in two phases, utilising the HPTLC technique. The first phase involved isolating sucralose from low-calorie *basundi*, while the second phase focused on quantifying it through the HPTLC technique as followed by George et al. 2010.

Isolation of sucralose

The isolation procedure involved ultra-sonication (to break down aggregates) (Fig. 3), Carrez clarification (to remove proteins), and filtration for the removal of fat and sample matrix. It was conducted according to the methodology utilised by George et al. (2010) for estimating sucralose in *lassi*. For this, 10 g of low-calorie *basundi* stored at $30 \pm 1^\circ\text{C}$ was taken in a 25 mL beaker, followed by ultrasonication at $40 \pm 1^\circ\text{C}$ for 20 min. Afterwards, the solution was then cooled to room temperature ($30 \pm 1^\circ\text{C}$), and 2 mL each of Carrez solutions no. 1 and no. 2, along with 1 mL of methanol, were added. The solution was allowed to stand at room temperature for 10 min before being filtered using filter paper Whatman no. 1. The resulting filtrate, containing sucralose, was then utilised for HPTLC analysis.

Quantification of sucralose using the HPTLC technique

The quantitative analysis of sucralose was performed on an aluminium-backed HPTLC silica gel 60 F₂₅₄ (Kiesel gel 60 F₂₅₄) plate, following the methodology as suggested by George et al. 2010. The separation of sucralose from solvent (mobile phase)

was performed in the developing chamber and took 15 min (approximately). The plate was dried using an air blower and then sprayed with 15% (v/v) sulphuric acid and further heated at $120 \pm 1^\circ\text{C}$ for 10 min in the hot air oven. At this temperature, sucralose appeared as a charred spot.

Ten microliters of the filtrate were applied to the HPTLC plate at a distance of 1-1.5 cm from the plate’s sides, which had been previously activated for 30 min in the hot air oven at $100 \pm 1^\circ\text{C}$. The spots applied were dried simultaneously using a hot air

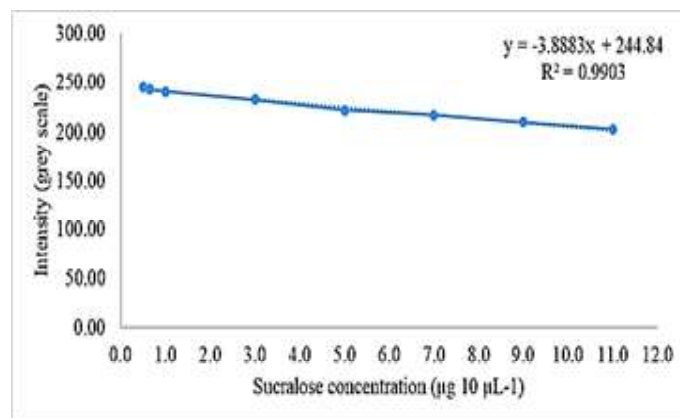


Fig. 2. Standard curve for sucralose

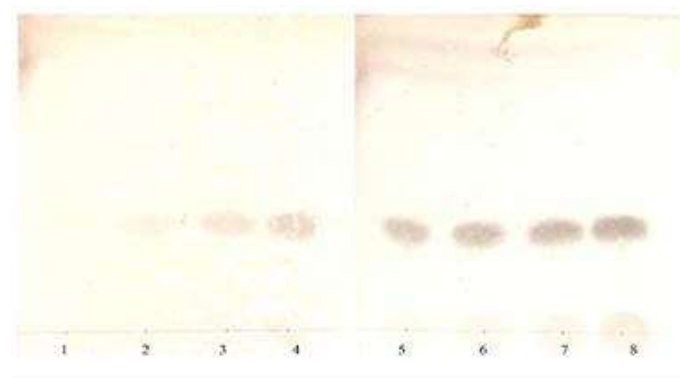


Fig. 3. Chromatogram at different concentrations of standard sucralose solution

Table 1: Effect of heat treatment on low-calorie *basundi* packaged in sterilisable PP containers

10% stabilizer solution (mL)	0.0	0.0	0.2	0.4	0.6	0.8	1.0
Distilled water (mL)	0.0	1.0	0.8	0.6	0.4	0.2	0.0
	Disodium phosphate						
115 °C / 5 min	+	+	-	-	-	-	-
115 °C / 10 min	+	+	-	-	-	-	-
121 °C / 5 min	+	+	+	+	+	+	+
	Trisodium citrate						
115 °C / 5 min	+	+	-	-	-	-	-
115 °C / 10 min	+	+	-	-	-	-	-
121 °C / 10 min	+	+	+	+	+	+	+

blower for 10-15 s. The plate was developed in a vertical chamber containing the reagents, viz., dichloromethane and methanol in the ratio (4:1), as shown in Fig. 4. The operation continued until a distance of 1 cm from the top edge of the plate. The separation process took 15 min (approximately). Then the plate was taken out from the vertical chamber and dried again using a hot air blower for 10-15 s. After this, the plate was sprayed with 15% methanolic sulphuric acid (v/v) and dried in a hot air oven maintained at 120±1 °C for 10 min. The sucralose appeared as a charred spot on the plate. A photographic image was created by scanning the plate with charred spots for further processing, as shown in Fig. 3. The intensity of these spots was measured on a grayscale basis using Image J 1.52a software, and the concentration was determined by plotting the intensity value in the standard sucralose curve.

Design of experiment

The pilot sterilisation test was conducted to ascertain the heat stability of low-calorie *basundi* at 115°C and 121°C for 5 and 10 min. The test determined the amount of stabiliser (disodium phosphate and trisodium citrate) required to enhance the heat stability of the low-calorie *basundi* (Table 1). The product heated at 115±1 °C for 5 min and stored at 30±1 °C was found acceptable for 30 days after being packaged in a sterilisable PP container, based on sensory, physicochemical, and microbiological analysis in storage studies. The heating effect on sucralose stability was determined at an interval of 15 days using the HPTLC technique. The amount of sucralose in high-temperature processed low-calorie *basundi* was compared before manufacture and after storage.

Statistical analysis

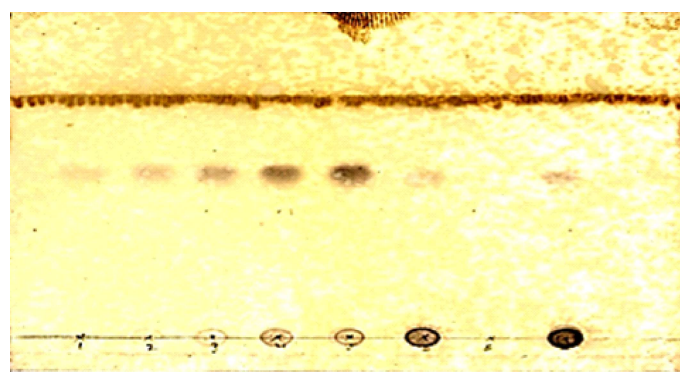
The data were subjected to a one-way analysis of variance (ANOVA) and/or student’s test to check the differences in means of the responses at a 5% level of significance using SPSS, IBM statistics (version 23).

Results and discussion

In a pilot sterilisation test, the 10 % solution of disodium phosphate or trisodium citrate was added to 170 g of low-calorie *basundi*.

Heat stability of low-calorie basundi

The test resulted in the coagulation of low-calorie *basundi* even at 1 mL of stabiliser solution at 121±1 °C for 5 min. It is due to the low pH (6.16) and high temperature that caused intense brown colour (due to Maillard reaction), and the cooked flavour was developed, which indicated sulfhydryl groups. The sulfhydryl groups had formed due to the breakdown of the calcium-casein complex. Thus, the coagulation and presence of cooked flavour indicated the instability of low-calorie *basundi* at 121±1 °C for 5 min. The coagulation and cooked aroma are an indication of



° Spots labelled viz., 1, 2, 3, 4, 5, S, 6, and 7 contain different sucralose quantity

Fig. 4. Chromatogram of sample isolates of high-temperature processed (115±1 °C for 5 min) low-calorie *basundi* on 15th day stored at 30±1 °C

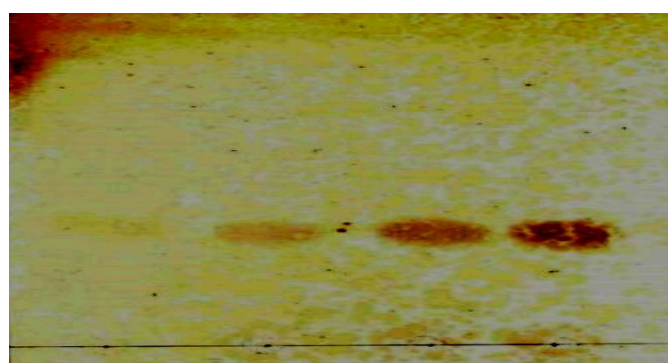


Fig. 5. Chromatogram of sample isolates of high-temperature processed (115±1 °C for 5 min) low-calorie *basundi* on 30th day stored at 30±1 °C.

Table 2: The intensity of charred spots at different concentrations of standard sucralose solution

S. No.	Concentration (µg/10 µL)	Intensity (grayscale)
1	0.50	244.890
2	0.65	242.914
3	1.00	240.497
4	3.00	232.662
5	5.00	222.166
6	7.00	217.470
7	9.00	210.473
8	11.00	203.161

reduced heat stability of milk (O’connell and Fox 2011). Therefore, the hypothesis of processing low-calorie *basundi* at 121 °C for shelf-life extension was rejected. Based on the

Table 3: The quantity of sucralose in a charged sample (filtrate) derived from low-calorie *basundi* and the intensities of the charged spots generated on the HPTLC plate on the 15th day^a

Spot. No.	Sample applied (µL)	Intensity (grayscale)			Average intensity (grayscale)	Sucralose (µg)			Average sucralose (µg)
		I ₁	I ₂	I ₃	I _{avg}	S ₁	S ₂	S ₃	S _{avg}
1	13	238.30	237.31	237.30	237.64±0.574	1.68	1.94	1.94	1.85±0.147
2	15	236.52	236.52	236.53	236.53±0.006	2.14	2.14	2.14	2.13±0.001
3	20	233.76	233.76	233.75	233.76±0.006	2.85	2.85	2.85	2.85±0.002
4	25	230.89	230.90	230.93	230.91±0.021	3.59	3.59	3.58	3.58±0.005
5	30	228.21	228.21	228.20	228.21±0.005	4.28	4.28	4.28	4.28±0.001
S	10	239.30	239.30	239.30	239.3±0.001	1.43	1.43	1.43	1.43±0.001
6	5	242.07	242.07	242.07	242.07±0.002	0.71	0.71	0.71	0.71±0.001
7	7	240.96	240.96	240.96	240.96±0.001	1.00	1.00	1.00	0.99±0.001

Sucralose quantity (µg) in 10 µL filtrate	
Spot No.	Sucralose (µg)
1	1.425 ^a
2	1.425 ^a
3	1.425 ^a
4	1.434 ^b
5	1.426 ^a
6	1.425 ^a
7	1.425 ^a

^a Data are means (n = 3) ± SD; means within the same column with different superscript letters differ significantly (P<0.05)

Table 4: The quantity of sucralose in a charged sample (filtrate) derived from low-calorie *basundi* and the intensities of the charged spots generated on the HPTLC plate on the 30th day.^b

Spot. No.	Sample (µL)	Intensity (grayscale)			Average intensity (grayscale)	Sucralose (µg)			Average sucralose (µg)
		I ₁	I ₂	I ₃	I _{average}	S ₁	S ₂	S ₃	S _{average}
1	0	248.100	248.121	248.147	248.123±0.020	0	0	0	0
2	2	243.730	243.731	243.729	243.730±0.001	0.285	0.285	0.286	0.285±0.001
3	12	238.251	238.250	238.251	238.250±0.001	1.695	1.696	1.695	1.695±0.000
4	22	232.656	232.646	232.655	232.652±0.006	3.134	3.136	3.134	3.134±0.001

Sucralose quantity (µg) in 10 µL filtrate	
Spot. No.	Sucralose (µg)
1	0 ^a
2	1.426 ^b
3	1.424 ^b
4	1.425 ^b

^b Data are means (n = 3) ± SD; means within the same column with different superscript letters differ significantly (P<0.05)

physicochemical properties, specifically the color attributes viz., *L**, *a**, *b** recorded at 70.13±0.01, 6.74±0.01, and 23.76±0.0 respectively, along with the sensory attributes evaluation conducted using a 9-point hedonic scale, which included flavor (7.93±0.15), color and appearance (7.77±0.12), consistency (8.05±0.05), and overall acceptability (7.89±0.06), the product

containing disodium phosphate (0.012%) heated to 115±1 °C for 5 min received the highest score, resulting in its selection for storage studies.

Checking of sucralose stability

The sucralose added to the milk of batch 2.50 kg for the manufacture of 1.14 kg low-calorie *basundi* was 65 ppm w.r.t initial milk (Singh et al. 2022). Therefore, 10 g (equivalent to 10 mL of filtrate) of the finished product had sucralose 1425 µg, or 10 µL of filtrate contained 1.425 µg of sucralose.

The HPTLC technique was used to determine the concentration of sucralose in the high-temperature processed (115 ± 1 °C for 5 min) low-calorie *basundi* at intervals of 15 days. After quantification, the filtrate showed no decrease in sucralose concentration on the 15th day. Before the intensity measurement of charred spots, the image of the plate (size, 1.8 MB; resolution, 1596×1156 pixels), was converted from RGB color mode to 8-bit grayscale. The concentration was determined by assessing the intensity of charred (fluorescent) spots formed on the HPTLC plate after running different quantities of the filtrate viz., 13, 15, 20, 25, 30, 10, 5, and 7 µL, as shown in Fig. 4. The amount of filtrate applied, intensity observed, and sucralose quantified for low-calorie *basundi* is tabulated in Table 2. The mean of the sucralose concentrations obtained by plotting the intensity values for each spot in the regression equation of the standard curve was 1.425 µg for a 10 µL filtrate. This sucralose concentration was equivalent to the amount of sucralose initially added to the milk (65 ppm of milk). Thus, it confirmed the stability of sucralose in low-calorie *basundi* at HTP upon storage at 30 ± 1 °C in PP containers for 15 days. Also, the time required for manufacturing after the concentration of total milk solids (TMS) to 20% w/w, and the addition of sucralose (65 ppm of milk) to achieve the final 36% TS (w/w) was 17 min. Therefore, it also confirmed sucralose stability at 90 ± 1 °C for 17 min. The reduction in pH of low-calorie *basundi* from 6.16 (1st day) to 6.12 (15th day) supported the stability of sucralose even at high temperatures in low-calorie *basundi*.

On further investigation, the product on the 30th day, no change or decrease in the sucralose was noticed. The different quantities of sample applied on the HPTLC plate resulted in different intensities of the charred spots as shown in Fig. 5. The image of the plate had a size, 140 kB and resolution, 333×432 pixels. These intensity values on plotting in the regression equation resulted in the average sucralose concentration of 1.426 µg for a 10 µL filtrate. No significant difference was observed among the sucralose concentrations determined on the 1st, 15th, and 30th days (all confirmed the same value). Hence, it confirmed 1425 µg of sucralose in 10 g low-calorie *basundi* (pH 6.07) on the 30th day, equivalent to 65 ppm of milk initially.

No decrease in sucralose content was observed after high-temperature processing (HTP) of low-calorie *basundi* at 115 ± 1 °C for 5 min, indicating its stability at or below this temperature, likely due to its chemical structure. However, processing at temperatures above 115 °C could yield different results. Bannach

et al. (2009) also reported the similar results who studied thermal stability of sucralose using thermogravimetry and differential thermal analysis (TG-DTA), differential scanning calorimetry (DSC) and infrared spectroscopy.

Conclusions

The study showed an application of HPTLC technique to analyse the thermal degradation of sucralose heated to 115 ± 1 °C for 5 min. The developed HPTLC plate for low-calorie *basundi* showed the intensity values of 239.295 for the 10 µL filtrate on the 15th day, and 238.250 for the 12 µL filtrate on the 30th day (based on grayscale). Plots on the standard regression equation and statistical analysis confirmed 1.425 µg sucralose for the 10 µL sample. This was equal to the amount (65 ppm of milk) of sucralose initially added to milk for the manufacture of low-calorie *basundi*, i.e., 1425 µg sucralose in 10 g of product. The sucralose in low-calorie *basundi* was found stable for HTP (115 °C for 5 min), at 90 ± 1 °C for 17 min, and storage (30 ± 1 °C) for 30 days. The information explored from this study is extremely valuable for the HTP, manufacture, and shelf-life enhancement of low-calorie heat desiccated milk products containing sucralose.

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