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Suitability of aspartame for preparation of low calorie *Karadkheer*

AR Walale, MR Patil and HM Gawande

Abstract
Karadkheer was prepared by blending the safflower milk with milk and other ingredients as per standard method. Low calorie *karadkheer* was prepared by replacing 15%, 30%, 45% and 60% sugar with aspartame and stored at 10 ºC. *Karadkheer* prepared with replacement of sugar up to 30% with was best accepted during storage which possessed the same desirable sweetness and sensory attributes even after three days of storage. Storage studies revealed that aspartame sweetened *karadkheer* resembled the control *karadkheer* in retaining the sensory profile, but showed rise in acidity and microbial load. HPLC analysis of aspartame sweetened low calorie *karadkheer* samples showed no degradation of aspartame up to 1st day of storage but on 2nd and 3rd days of storage 2, 5 – diketopeprazine and L-phenylalanine were identified in all the samples containing aspartame.

Keywords: *Karadkheer*, aspartame, artificial sweetener, low calorie

Introduction
Aspartame is in use as a sweetener and as a table-top sweetener for more than 30 years in many countries all over the world. It is a dipeptide methyl ester of L-aspartyl-L-phenylalanine. It is an odourless, white crystalline powder which has a clean, sweet taste. Aspartame is about 200 times sweeter than sucrose. Aspartame is stable in the dry state and in frozen products. However, when stored in liquids at more than 30°C, it progressively converts into diketopiperazine, which is partially degraded into methanol, aspartic acid and phenylalanine. These transformations result in the loss of sweetness (www.greenfacts.org, 2018) [16]. The Government of India has permitted the use of aspartame in some food items. According to a Food Safety and Standards Regulations, 2011(FSSR, 2011) [2], the use of artificial sweeteners has been allowed in food items as per the limits prescribed and under proper label declarations. The traditional dairy sweets and desserts have great cultural relevance and market in India. Presently around 150 types of milk based sweet meats are available in the country. One such traditional cereal based popular dessert is *kheer*. *Karadkheer* a value added version of *kheer*, is a rural indigenous milk product prepared in Maharashtra state of India. It is prepared from milk, safflower milk prepared out of safflower (*Carthamus tinctorius* L.) extract, sugar and basmati rice.

Aspartame is added to more than 1200 food products including dairy products. Long term storage and heat stability are important factors for the use of aspartame in many food products. Artificial sweeteners have been found to have different responses to heat and pH (George *et al.* 2006) [9]. Since the application of aspartame in indigenous dairy products is new, qualitative as well as quantitative information on sweetener's degradation in dairy system is required. According to FSSR (2011) [2], the use of aspartame has also been allowed in traditional sweets like, *halwa, gulabjamun, khoya burfi, rasogulla,* and other sweets.

Replacement of sugar in *karadkheer* with artificial sweeteners like aspartame not only fulfills the demand of low calorie product but can also make it suitable for such people, who are health conscious in respect to diabetes, obesity, weight control, and dental caries. Therefore, the present study has been undertaken to identify the most appropriate inclusion rate for aspartame to prepare an organoleptically acceptable *karadkheer* and to investigate the stability of aspartame in the product during storage.

Material and Methods

Sweetener and its standard degradation products

Aspartame powder (Himedia, Mumbai). Aspartame degradation products standards: 2, 5-diketopiperazine, L-phenylalanine (Sigma-Aldrich, Lovfs, MO, USA).

Chemicals and Media

Sulfuric acid, Iso-amyl alcohol, potassium sulphate, copper sulphate, sodium hydroxide, boric acid, hydrochloric acid, phenolphthalein indicator, standard buffer tablet of pH 4 and pH 7, Plate count agar, Potato dextrose agar (AR Grade, Himedia laboratories, Mumbai), sodium chloride, ethyl alcohol, diethyl ether, petroleum ether, zinc sulphate, potassium ferrocyanide, dipotassium hydrogen phosphate, potassium dihydrogen phosphate (AR Grade, Molychem, Mumbai), acetonitrile, methanol, water (HPLC Grade, Molychem, Mumbai) Filter papers: Whatman No.42 (1.5 µm). Carrez solution No.1, 3.6 g of potassium ferrocyanide dissolved in 100 ml HPLC grade water. Carrez solution No. 2, 7.2 g of zinc sulphate dissolved in 100 ml HPLC grade water. Mobile phase: 0.02 M phosphate buffer pH (3.5): acetonitrile (80:20).

Standard Solution of Aspartame and degradation product

Ten mg of sweetener and its degradation products were dissolved separately in 10 ml of mobile phase to get stock standard solutions, with a concentration of 1 mg/ml. One hundred µl of stock standard solution were pipetted into separate 10 ml volumetric flasks and volume was made up to mark with mobile phase to get standard solution of concentration 10 ng/µl. (also 100 µl from each stock standard solution (1 mg/ml) of aspartame and its degradation products, 2,5 diketopiperazine, L-phenylalanine were pipetted into 10 ml volumetric flasks and volume was made up to the mark with mobile phase to get mixture of each component as in the mixed solution of concentration of 10 ng/ µl).

Equipments

Kelplus digestion and distillation assembly for protein estimation was supplied by Pelican Instruments, Chennai; Millipore type membrane filter (Z-37) by ASGI, India; High speed cooling centrifuge C-24 BL REMI by Remielectrotechnik Ltd., Mumbai and HPLC system by Dionex (UHPLC 3000 system, UV detector).

Preparation of *Karadkheer*

Fresh, white colour safflower seeds and high quality basmati rice were procured from local market. Low calorie *karadkheer* sweetened with aspartame was prepared using the method described by Sarode (2004) [13], with slight modification. The preparation of *kheer* was carried out in two different phases. In the initial phase, the safflower milk was prepared from safflower seeds extract by method of Maske (1997) (Figure 1) and in later phase the *kheer* was prepared by blending the safflower milk with milk in 40:60 ratio (Figure 2). A control sample of *karadkheer* was also prepared using sugar and milk ('Rajhans' homogenized toned milk) procured from the local market and following the same method.

The method required sugar @ 9% of safflower milk and milk blend. *Karadkheer* prepared by using sugar @ 9% was considered as control and on the basis of sugar equivalence sugar was replaced at different levels with equivalent sweetness level of aspartame. Treatment and levels were as follows;

T_1= 85% sugar + aspartame equivalent to 15% sugar.
T_2= 70% sugar + aspartame equivalent to 30% sugar.
T_3= 55% sugar + aspartame equivalent to 45% sugar.
T_4= 40% sugar + aspartame equivalent to 60% sugar.

Aspartame is unstable if subjected to prolonged heat treatment and therefore cannot be used in baking or cooking unless added at the end of the cooking process (Kroger *et al*., 2006) [11]. Aspartame could have degraded at high temperature attained while preparing the *karadkheer*, hence, it was added at the end stage when *karadkheer* was cooled at room temperature. The product was homogenously mixed to ensure uniform distribution of aspartame in the product.

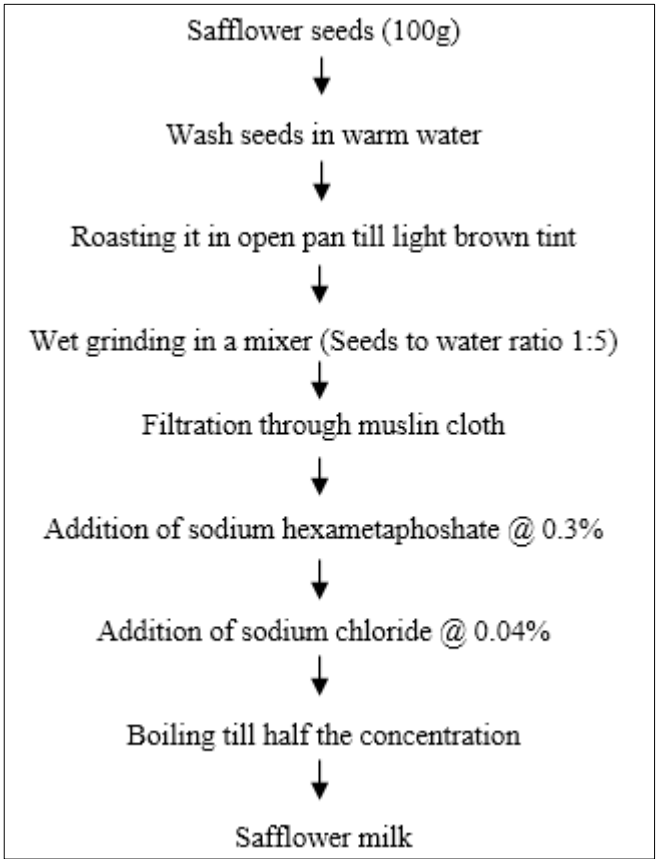

Fig 1: Flow chart for preparation of safflower milk

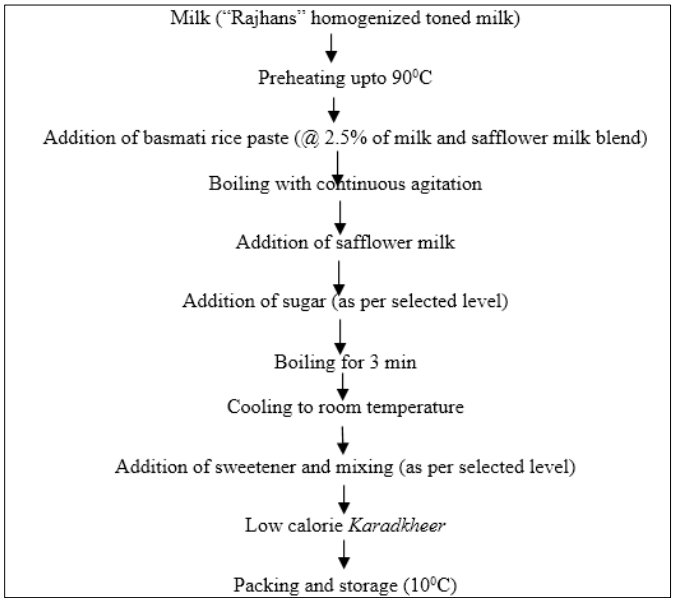

Fig 2: Flow diagram for preparation of low calorie *karadkheer*

Sample preparation and HPLC analysis

Sample preparation procedure used for isolation (Figure 3) of sweetener from *karadkheer* was based on method of BSEN: 12856 (1999). Weights of aspartame sweetened samples for different treatments taken for sample preparation are given in Table 1. Calculated sample of low calorie *karadkheer* was taken in 100 ml beaker and added with 50 ml of HPLC grade water. Solution was transferred to a 100 ml volumetric flask. Six ml of Carrez solution No. 1 was added and mixed followed by addition of 6 ml of Carrez solution No.2 to the solution. The solution was shaken vigorously and allowed to stand at room temperature for 10 min. After dilution upto the mark with mobile phase, filtration was carried out using a Whatman No. 1 filter paper through sintered funnel. Filtrate was centrifuged to clear supernatant and was used for HPLC analysis during storage. HPLC analysis of reference standards of aspartame, its degradation products and sample isolates from *karadkheer* were performed under standardized conditions. Reverse phase HPLC analysis of aspartame its degradation products and sample isolates from *karadkheer* were performed over C_{18} column at UV 200 nm. Mobile phase was used at a flow rate of 1 ml/min and run time of 30 min.

Table 1: Weight of aspartame sweetened *karadkheer* for HPLC analysis

Treatment	Aspartame sweetened samples (g)
T1	38.37
T2	15.82
T3	8.87
T4	6.48

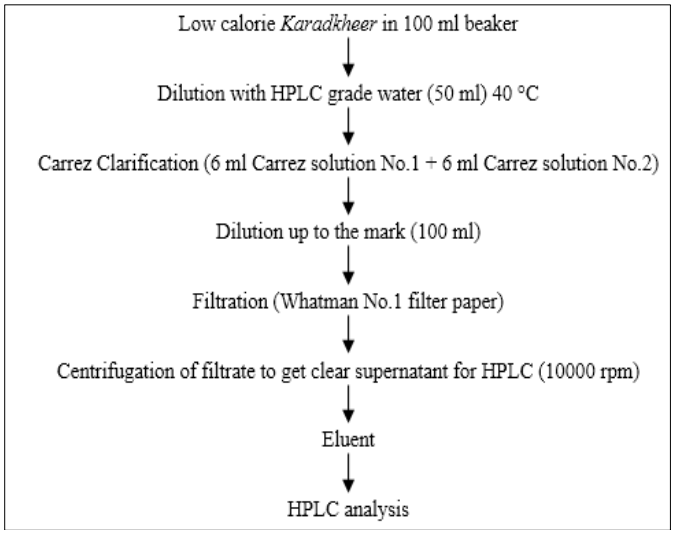

Fig 3: Flow chart for isolation of aspartame from *Karadkheer*

Chemical composition of *karadkheer*

Aspartame sweetened *karadkheer* and control sample composition was analyzed for moisture and fat (IS: SP Part XI, 1981), total protein, ash and pH (AOAC, 1990).

Storage and analysis of *karadkheer*

Control and artificially sweetened samples of *karadkheer* were packed in polypropylene cups and stored at refrigerated temperature (10 °C). The samples were analyzed from first to third day of storage for titratable acidity, pH, Standard Plate Count and Yeast and Mould using standard method (IS: SP Part XI, 1981). Incubation of the plates was carried out at 37 °C for 48 hrs. and 30 °C for 3-5 days respectively.

Sensory Evaluation

The samples of *karadkheer* were evaluated for the sweetness, colour and appearance, body & texture and overall acceptability by a panel of 5 judges from the faculty of College of Dairy Technology, Warud (Pusad) using 9-point hedonic scale score card (Amerine *et al*., 1965) [4].

Statistical analysis

In all experiments, one-way analysis of variance (ANOVA) with a subsequent least significant difference (LSD) test was applied for multiple sample comparison. This was done to test for any significant differences ($P<0.05$) in the mean values of all the treatments as described by Snedecor and Cochran (1989) [14]. Data from three replications of each experiment were analyzed for statistical analysis.

Results and Discussion

Effect of aspartame on chemical composition of *karadkheer*

Replacement of 15%, 30%, 45% and 60% of the total sugar in *karadkheer* with equivalent quantity of aspartame resulted into non-significant difference ($P<0.05$) in fat, protein, ash and pH with respect to control (Table 2). However, samples sweetened with aspartame noticed slight increase in moisture content with increase in aspartame replacement of sugar in respect to control. The results for compositional parameters of aspartame sweetened *karadkheer* were in accordance with results reported by Sarode (2004) [13] for *karadkheer*, De *et al.* (1976) [6] for *kheer* prepared without safflower extract and Mathur *et al.* (1985) for *kheer* prepared with 10% basmati rice and 15% sugar.

Storage related changes in *karadkheer*

Table 2: Chemical composition of aspartame sweetened *karadkheer*

Parameters	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	pH
Control	63.55 ± 0.77^{d}	7.68 ± 0.09^{a}	5.58 ± 0.02^{a}	0.78 ± 0.06^{a}	5.81 ± 0.09^{a}
T1	64.28 ± 1.00^{cd}	7.86± 0.14^{a}	5.51 ± 0.08^{a}	0.82± 0.01^{a}	5.70± 0.16^{a}
T2	66.11 ± 2.14^{bc}	7.80± 0.12^{a}	5.57 ± 0.08^{a}	0.80± 0.02^{a}	5.72± 0.10^{a}
T3	67.97 ± 1.25^{ab}	7.91± 0.19^{a}	5.69 ± 0.05^{a}	0.82± 0.02^{a}	5.75± 0.09^{a}
T4	69.36 ± 0.91^{a}	7.79± 0.13^{a}	5.64 ± 0.11^{a}	0.80± 0.03^{a}	5.76± 0.07^{a}

All scores are average of three replications. The superscripts a, b, c and d in each column indicate significantly different means at $P < 0.05$. Data are presented as Means ± S.D

Sensory profile

Sensory analysis (Table 3) revealed that T_2 was best accepted among all the treatments studied during storage. The sweetness scores of all the treatments and control showed decrease in sweetness during storage. The score of T_2 was superior to remaining treatments as well as control. Similar results were observed by Arora *et.al*. (2010) [3] for aspartame sweetened *burfi*. The colour scores of all the treatments were at par during period of storage and resembled control. The appearance score of all the treatments including control showed significant difference in the fresh and stored samples. Control and T_2 samples were acceptable up to the 3rd day of storage, but there was significant reduction in score than fresh sample. The body and texture score of control was highest

and at par with T_1 and T_2 samples. Samples T_3 and T_4 were significantly lower ($P<0.05$) from control, T_1 and T_2. Samples of T_1, T_3 and T_4 showed decreasing scores up to 3rdday of storage. *Karadkheer* become thicker during storage could be due the presence of sugar and starch in the rice and hence, resulted in lower scores. The T_2 sample was best accepted and at par with control for body and texture scores during storage. The flavour scores of sample T_3 and T_4 were significantly different ($P<0.05$) from control, T_1 and T_2. Control and T_2 samples were acceptable up to the 3rd day of storage, but there was significant reduction in score than fresh sample. The score for all of the treatments reduced significantly from 2nd day of storage and the product was less accepted mainly due to development of bitter off flavor.

The overall acceptability score of the T_2 was highest among all the treatments and resembled control upto the end of storage. The T_1 sample also showed the acceptable score up to 3rd day but scored lower than control and T_2. While the scores for the T_3 and T_4 sample were significantly lower from initial day up to the last day of storage.

It is evident that the sensory scores of T_2 for all sensory attributes were at par with control and more than control in some cases, which indicates its acceptability on the sensory ground. It was also observed that the rate of decline in the sensory scores of T_2 was more than that of control during the storage. Similar results were observed with aspartame sweetened *burfi* (Arora *et al.,* 2010) [3], *kalakand* (Gawande *et al.,* 2015) [8] and carrot *halwa* (Vairagade *et al.,* 2017) [15] during storage.

Table 3: Changes in quality of aspartame sweetened *karadkheer* during storage at 10 ºC

Particulars of *Karadkheer*	Storage Days		
	1	2	3
Sensory Attributes			
Sweetness			
Control	7.40 ± 0.51^{ab}	7.36 ± 0.62^{a}	7.07 ± 0.46^{ab}
T1	7.53 ± 0.74^{ab}	7.43 ± 0.63^{a}	7.07± 0.80^{ab}
T2	7.80 ± 0.77^{a}	7.64 ± 0.62^{a}	7.33 ± 0.98^{a}
T3	7.07± 0.80^{b}	6.71 ± 0.70^{b}	6.53 ± 0.92^{bc}
T4	6.33 ± 0.90^{c}	6.36 ± 0.83^{b}	5.93 ± 0.96^{a}
Color			
Control	7.47 ± 0.64^{a}	7.29 ± 0.62^{a}	7.20 ± 0.68^{a}
T1	7.40 ± 0.51^{a}	7.29 ± 0.49^{a}	7.13 ± 0.64^{a}
T2	7.53 ± 0.64^{a}	7.43 ± 0.09^{a}	7.27 ± 0.80^{a}
T3	7.47 ± 0.64^{a}	7.29 ± 0.58^{a}	7.13 ± 0.74^{a}
T4	7.20 ± 0.68^{a}	7.14 ± 0.80^{a}	6.67 ± 0.72^{a}
Appearance			
Control	7.67 ± 0.62^{a}	7.50 ± 0.64^{a}	7.47 ± 0.64^{a}
T1	7.20 ± 0.18^{ab}	7.14 ± 0.74^{ab}	7.07 ± 0.46^{ab}
T2	7.67 ± 0.62^{a}	7.64 ± 0.51^{a}	7.40 ± 0.74^{a}
T3	7.20 ± 0.77^{ab}	7.07 ± 0.74^{ab}	7.07 ± 0.70^{ab}
T4	6.93 ± 0.70^{b}	6.71 ± 0.82^{b}	6.67 ± 0.72^{b}
Body and texture			
Control	7.40 ± 0.63^{a}	7.43 ± 0.63^{a}	7.13 ± 0.83^{a}
T1	7.20 ± 0.77^{a}	7.14 ± 0.64^{ab}	7.00 ± 0.76^{a}
T2	7.33 ± 0.90^{a}	7.43 ± 0.64^{a}	7.13 ± 0.92^{a}
T3	6.93 ± 0.88^{ab}	6.79 ± 0.77^{bc}	6.53 ± 0.83^{ab}
T4	6.47 ± 0.99^{b}	6.29 ± 0.74^{c}	6.20 ± 0.86^{b}
Flavor			
Control	7.40 ± 0.63^{a}	7.36 ± 0.51^{a}	7.33 ± 0.62^{a}
T1	7.20 ± 0.77^{a}	7.14 ± 0.74^{a}	7.00 ± 0.76^{ab}
T2	7.40 ± 0.63^{a}	7.29 ± 0.59^{a}	6.93 ± 0.88^{ab}
T3	7.00 ± 0.85^{ab}	6.50 ± 0.74^{b}	6.53 ± 0.99^{bc}
T4	6.53 ± 0.64^{b}	6.43 ± 0.64^{b}	6.13 ± 0.83^{c}
Overall Acceptability			
Control	7.53 ± 0.52^{a}	7.36 ± 0.49^{ab}	7.27 ± 0.70^{a}
T1	7.03 ± 0.77^{ab}	6.96 ± 0.72^{bc}	7.03 ± 0.72^{a}
T2	7.53 ± 0.74^{a}	7.57 ± 0.51^{a}	7.17 ± 0.99^{ab}
T3	7.00 ± 0.93^{b}	6.50 ± 0.74^{cd}	6.53 ± 0.83^{b}
T4	6.47 ± 0.52^{c}	6.43 ± 0.74^{d}	5.87 ± 0.83^{c}
Physicochemical Attributes			
pH			
Control	5.81 ± 0.09^{a}	5.79 ± 0.10^{a}	5.76 ± 0.10^{a}
T1	5.70 ± 0.16^{a}	5.69 ± 0.17^{a}	5.61 ± 0.14^{a}
T2	5.72 ± 0.10^{a}	5.71 ± 0.09^{a}	5.66 ± 0.05^{a}
T3	5.75 ± 0.09^{a}	5.73 ± 0.11^{a}	5.67 ± 0.05^{a}
T4	5.76 ± 0.07^{a}	5.75 ± 0.09^{a}	5.70 ± 0.09^{a}
Acidity (% LA)			
Control	0.066 ± 0.002^{a}	0.068 ± 0.009^{a}	0.085 ± 0.006^{a}
T1	0.063 ± 0.005^{a}	0.070 ± 0.004^{a}	0.080 ± 0.008^{a}
T2	0.065 ± 0.005^{a}	0.075 ± 0.004^{a}	0.083 ± 0.004^{a}

T3	0.064 ± 0.004[a]	0.077 ± 0.001[a]	0.092 ± 0.010[a]
T4	0.065 ± 0.003[a]	0.075 ± 0.003[a]	0.093 ± 0.009[a]
Microbiological Quality			
SPC (log cfu/g)			
Control	1.57 X 10^4 ± 1.01[e]	3.53 X 10^4 ± 3.24[e]	3.89 X 10^4 ± 0.73[e]
T1	2.06 X 10^4 ± 0.50[d]	3.23 X 10^4 ± 0.86[d]	3.52 X 10^4 ± 0.31[d]
T2	2.15 X 10^4 ± 0.40[c]	3.37 X 10^4 ± 0.57[c]	3.64 X 10^4 ± 0.50[c]
T3	2.53 X 10^4 ± 1.77[b]	3.43 X 10^4 ± 0.33[b]	3.99 X 10^4 ± 0.79[b]
T4	2.91 X 10^4 ± 0.55[a]	4.55 X 10^4 ± 1.61[a]	4.30 X 10^4 ± 0.89[a]
Yeast & Mold Count (log cfu/g)			
Control	0.40x10^4 ± 0.56[a]	0.51x10^4 ± 0.59[a]	0.33x10^4 ± 0.05[a]
T1	0.21x10^4 ± 0.11[b]	0.36x10^4 ± 0.24[b]	0.30x10^4 ± 3.53[b]
T2	0.15x10^4 ± 0.04[c]	0.30x10^4 ± 0.12[c]	0.24x10^4 ± 0.12[c]
T3	0.13x10^3 ± 0.02[d]	0.24x10^4 ± 0.04[d]	0.22x10^4 ± 0.09[d]
T4	0.10x10^4 ± 0.05[e]	0.21x10^3 ± 0.02[e]	0.22x10^4 ± 0.06[d]

All scores are average of three replications. The superscripts a, b, c and d in each column of each attribute indicate significantly different means at P < 0.05. Data are presented as Means ± S.D

Titratable acidity and pH

Low calorie *karadkheer* sweetened with aspartame and control observed slight but statistically non-significant (P>0.05) decrease in pH during storage (Table 3). Similar results were observed by Gawande (2006) [7] and Vairagade *et al.* (2017) [15] for pH of aspartame sweetened *burfi* and carrot *halwa* respectively. Continuous increase in titratable acidity was noted in both control as well as aspartame sweetened *karadkheer* samples during the storage (Table 3). It was observed that samples containing more quantity of aspartame had more titratable acidity but this effect was not statistically significant. This difference in titratable acidity might be due to the slight preservative effect of sucrose, which led to the retarded microbial growth (Gawande *et al*., 2015) [8].

Microbiological examination

Total plate counts and Yeast and Mould counts increased for aspartame sweetened products and control during storage. Total plate counts were higher (*P*<0.05) in aspartame sweetened product than their corresponding control throughout the storage period (Table 3). The incorporation of aspartame reduces concentration of sugar, lowering the preservation effect of sugar, ultimately leads to higher microbial counts. The results observed are in accordance with Total plate count observed for aspartame sweetened *burfi* (Arora *et al.*, 2010) [3], *kalakand* (Gawande *et al.*, 2015) [8] and carrot *halwa* (Vairagade *et al.*, 2017) [15]. However, Yeast and Mold counts of aspartame sweetened low calorie *karadkheer* samples were lower as compared to control samples (Table 3). Presence of higher sugar levels ultimately lead to significantly linear rise (P<0.05) in Yeast and Mould counts in control and all the treatments.

Stability of Aspartame

Aspartame, diketopiperazine and L-phenylalanine gave λ_{max} at 200 nm. Fig. 4 represents HPLC chromatogram of these three components at 200 nm under the standardized analytical conditions.

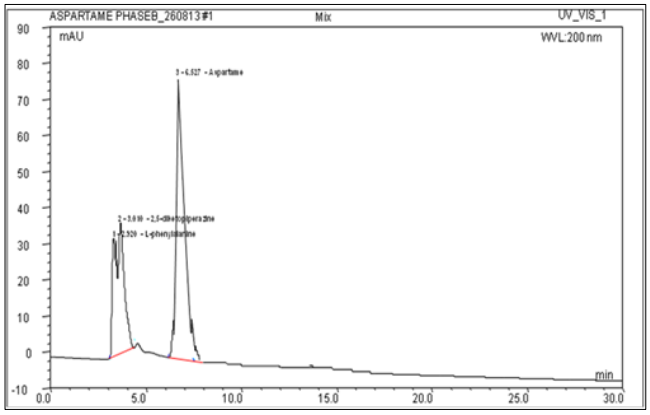

Fig 4: HPLC chromatogram of aspartame and its degradation products standards

Stability of aspartame in *karadkheer* during storage

HPLC chromatograms obtained during 1st to 3rd days of storage for aspartame sweetened *karadkheer* for all the treatments are represented in figure 5, 6 and 7 respectively. No peak other than aspartame can be seen in the chromatograms as shown in figure 5, establishing that aspartame was not degraded up to the 1st day of storage in all samples. HPLC chromatograms on 2nd and 3rd days as shown in figure 5 and 6 reported the degradation of aspartame. The degradation products identified were L-phenylalanine and 2,5-diketopiperazine. This might possibly be due to degradation of aspartame during storage as affected by changes in pH and temperature. Similar results were also observed in aspartame sweetened flavored milk (Gawande, 2006) [7] and carrot *halwa* (Vairagade *et al.*, 2017) [15] where aspartame was stable up to the 3rd day of storage but due to variation in pH and temperature it degraded at 5th day of storage.

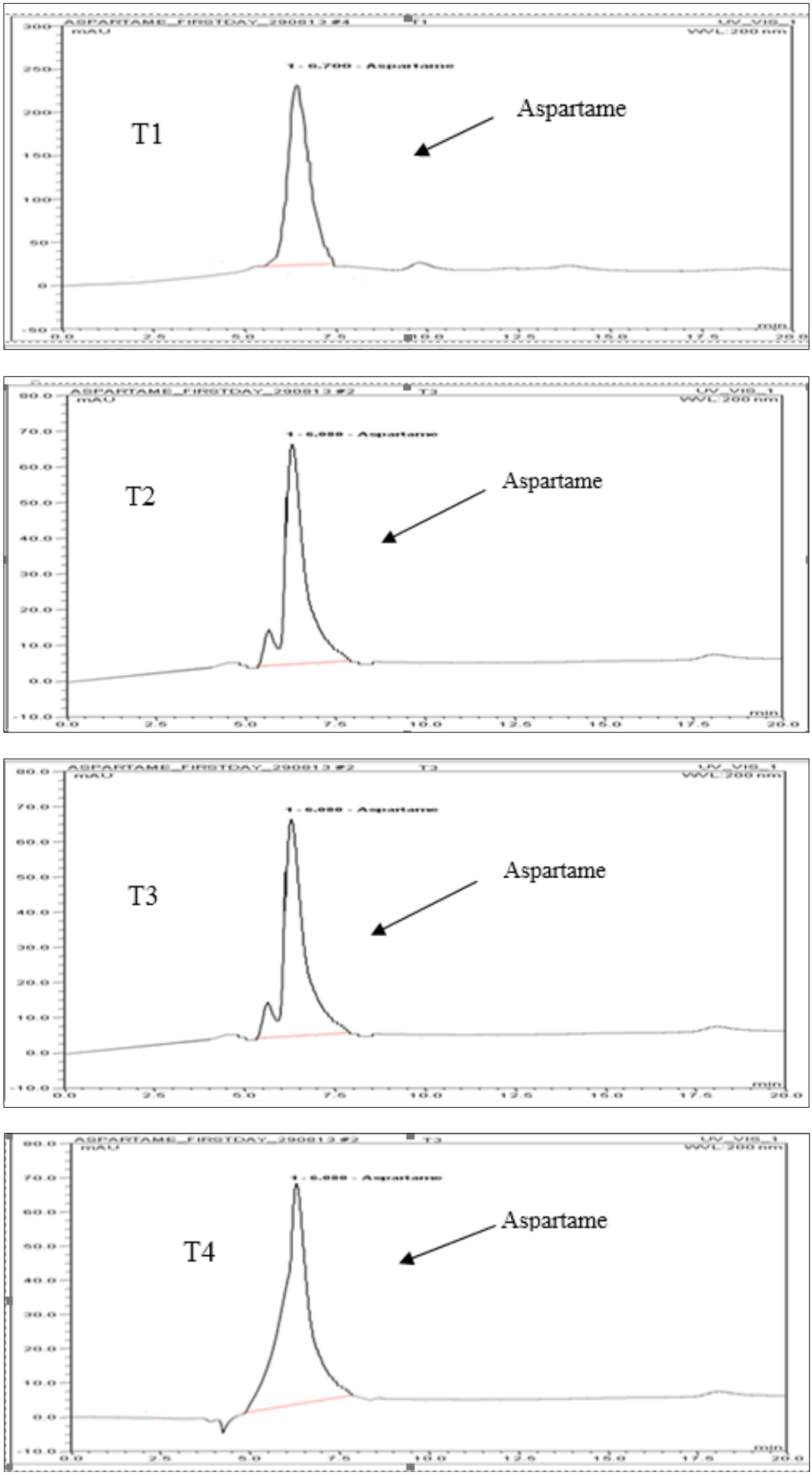

Fig 5: HPLC chromatograms of sample isolates of aspartame sweetened *Karadkheer* during 1st day of storage

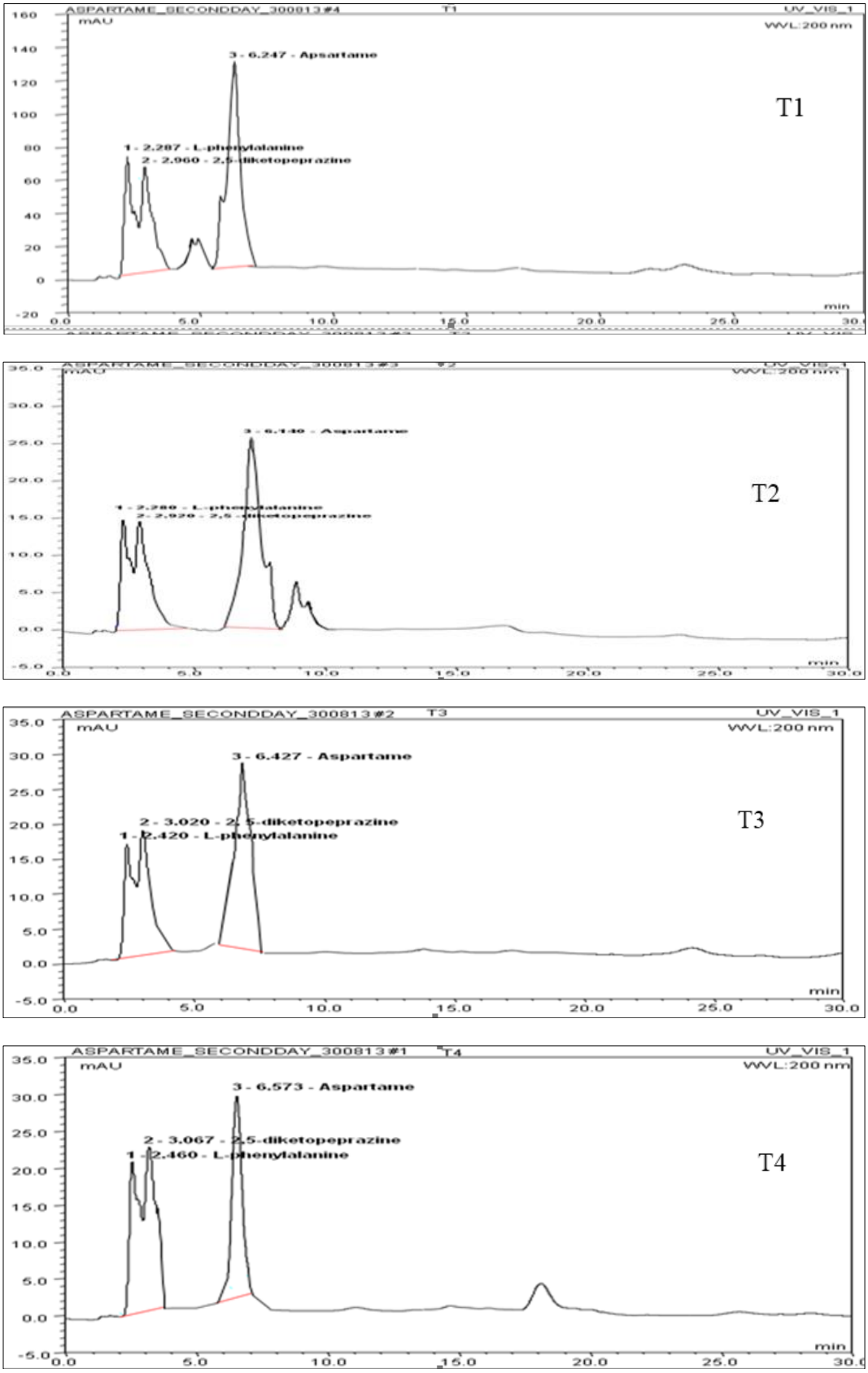

Fig 6: HPLC chromatograms of sample isolates of aspartame sweetened *Karadkheer* during 2nd day of storage

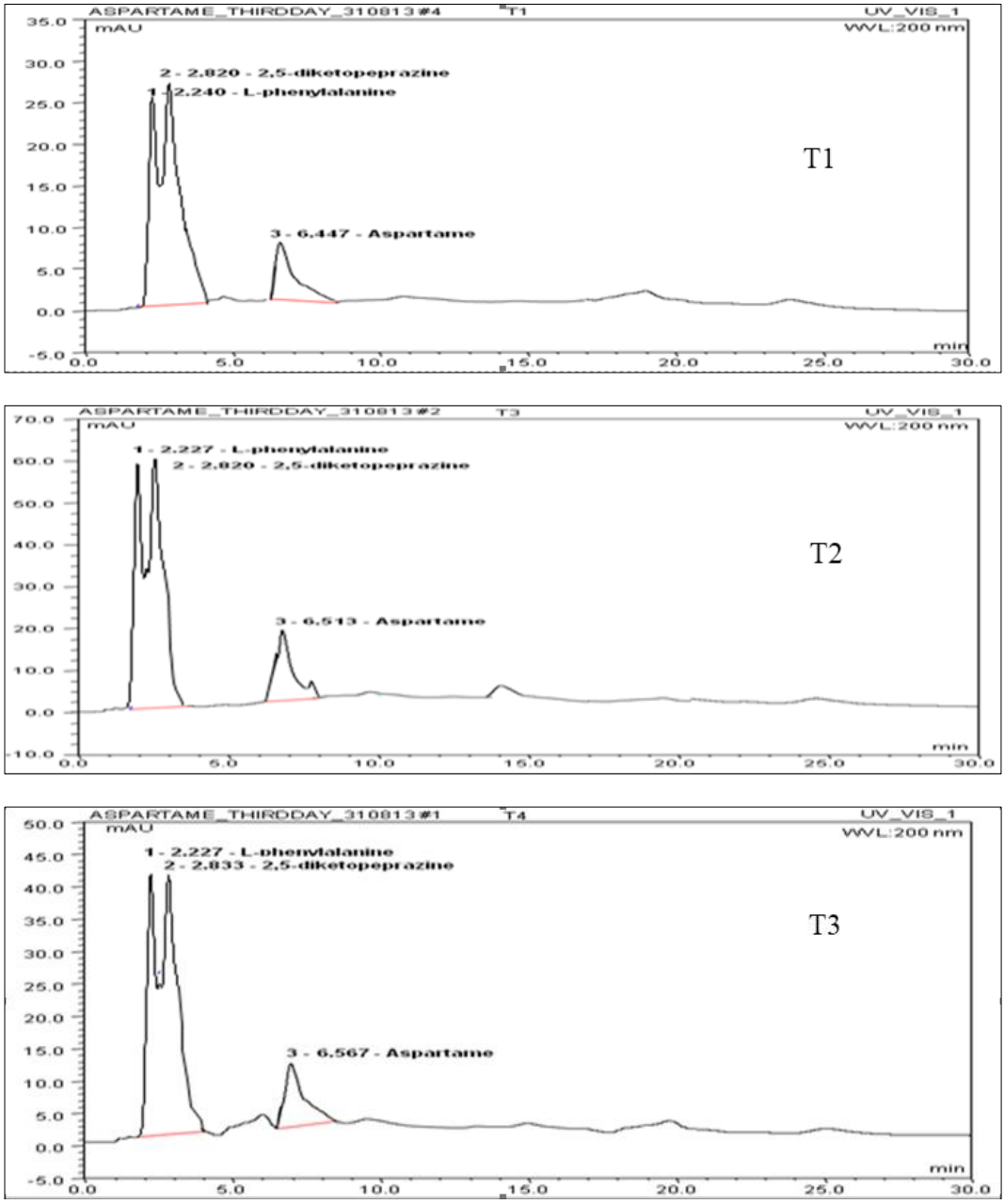

Fig 7: HPLC chromatograms of sample isolates of aspartame *Karadkheer* during 3rd day of storage

Conclusion

Sensory profile during storage study at 10 °C revealed that aspartame-sweetened *karadkheer* resembled the control *karadkheer* in retaining sweetness. Replacement of sugar level up to 30% was found optimum for the aspartame sweetened *karadkheer* on the basis of sensory evaluation. However, aspartame-sweetened *karadkheer* ranked lower than the control in sensory profile, acidity and microbial load. High-performance liquid chromatography analytical conditions were standardized for the separation of aspartame and its degradation product 2, 5-diketopiperazine and L-phenylalanine over a C_{18} column at UV 200 nm. HPLC analysis of aspartame sweetened *karadkheer* samples showed no degradation upto the 1st day but reported presence of aspartame degradation products, L-phenylalanine and 2, 5-diketopiperazine upon further storage in all samples.

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