Aspartame: safety and stability in *kalakand*

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Revised: 28 October 2013 / Accepted: 31 October 2013 / Published online: 16 November 2013
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Abstract Aspartame was used in the manufacture of *kalakand* instead of sucrose. Sensory evaluation revealed that aspartame when used in the preparation of *kalakand* at a level of 0.065 % scored the highest in terms of sweetness perception and resembled control. Aspartame sweetened *kalakand* possessed the same desirable sweetness, colour, body and texture/consistency and mouthfeel even after 7 days of storage at 6–8 °C. Significant increase in titratable acidity of control as well as aspartame sweetened *kalakand* was observed during storage. However, only a slight drop in pH was observed in all samples on storage. The titratable acidity was higher in aspartame sweetened products than the corresponding control samples. Lightness (L^*) was less in control samples with sucrose than the aspartame sweetened *kalakand* during storage. Total plate counts were higher in aspartame sweetened *kalakand* than its corresponding control throughout the storage period. Total plate counts increased linearly for both aspartame sweetened *kalakand* and control. A solid phase extraction method was standardized for the isolation of aspartame in *kalakand*. HPLC analytical conditions were standardized for separation of aspartame and its degradation products diketopiperazine and L-phenylalanine. HPLC analysis revealed that aspartame

did not degrade in *kalakand* during storage establishing its stability in these products.

Keywords Aspartame · Safety · *Kalakand* · Degradation

Abbreviations

UV	Ultraviolet
Kg	Kilogram
mL	Mililitre
ng	Nanogram
μL	Microlitre
ppm	Parts per million
cm	Centimeter
$\lambda_{\max}$	Absorption maxima
a_w	Water activity
nm	Nanometer
SD	Standard deviation
LSD	Least significant difference

Introduction

Aspartame (*N*-L- α -aspartyl-L-phenylalanine methyl ester), discovered in 1965, is a low-calorie sweetener with a sugar-like taste but is approximately 200 times sweeter than sucrose. It is a white dipeptide, crystalline in nature. It is unique among low calorie sweeteners; in that it is completely broken down by the body to its components—the amino acids, aspartic acid and phenylalanine, and a small amount of methanol (Mazur 1974). Aspartame is the most widely used artificial sweetener and has captured 50 % of the world market since its introduction in 1981. The risks of aspartame ingestion would be in the toxicity of its metabolism products. The safety issues that have been raised in the past about aspartame have included: (1) the possibility of toxicity from methanol, one of the breakdown products of aspartame; (2) elevations in plasma concentrations of phenylalanine and aspartic acid, which could result in increased transport

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of these amino acids into the brain, altering the brain's neurochemical composition; (3) the possibility of neuroendocrine changes, particularly increased concentrations in the brain, synaptic ganglia and adrenal medulla of catecholamines derived from phenylalanine and its hydroxylation product, tyrosine; and (4) a postulated link with epilepsy and brain tumours. All these areas have been addressed in the pre-1988 literature and in recent reviews (Meldrum 1993; Lajtha et al. 1994; Tschanz et al. 1996). Therefore, it is important to study the thermal behavior of foodstuffs because the kinetic factors, temperature and time, can provoke decomposition of components into new compounds. Chemical interactions can occur during industrial processing, which may necessitate the development of better methodologies for quality control applications.

Consumption of sweets is an integral part of Indian dietary system and diabetic-friendly traditional sweets are a new category for such products; the production of which is being contemplated by many enterprising manufacturers. With increased consumer interest in reducing sugar intake, food products made with sweeteners rather than sugar have become more popular. Recent change in legislation in India (PFA 2004) now permits the use of aspartame in sweets like *halwa*, *khoa*, *burfi*, *rasogolla*, *gulabjamun* and other milk products upto 200 ppm as per the limits prescribed under proper label declaration. This can lead to partial or whole replacement of sucrose in sweetened dairy products like *kalakand*.

Kalakand is one of the most popular *khoa*-based sweets having granular fused mass, held together in a loosely compact body with off-white to light caramel colour. *Kalakand* is a milk product where milk is coagulated with small quantities of citric acid followed by desiccation and addition of sugar to obtain a product with a granular texture, caramel flavour, and paste-like consistency. Replacement of sugar with aspartame in *kalakand* may result changes in its physicochemical attributes. Since application of aspartame in *kalakand* is new, qualitative and quantitative information on sweeteners degradation in dairy systems is required. No work has been done on the estimation and stability of aspartame in indigenous dairy products. Therefore, study was carried out to optimize the rate of addition of aspartame for obtain an organoleptically acceptable product and determine the stability of aspartame during storage.

Material and methods

Preparation of *kalakand*

Pooled buffalo milk was collected from the Experimental Dairy, NDRI, Karnal. The artificially sweetened *kalakand* was prepared according to the method of Suresh and Jha (1994) with slight modification (Fig. 1) for aspartame containing product. A control sample of *kalakand* was also prepared using sugar and citric acid procured from the local market.

Optimization of aspartame levels in *kalakand*

Aspartame levels were optimized by sensory evaluation of *kalakand* using the basis of its acceptance by a panel of eight judges on a 9-point hedonic scale score card. The levels of aspartame tried for *kalakand* were in the range of 0.045–0.075 % on 6 % sucrose equivalence milk basis.

Aspartame standards

Nutrasweet powder (Nutrasweet Company, Augusta, GA, USA) was used as aspartame standard and its degradation products standards were 2, 5-diketopiperazine, L-Phenylalanine (Sigma-Aldrich, Lovfs, MO, USA). The chemicals used in this study were of analytical grade reagent. All aqueous solutions were prepared using reverse-osmosis quality water produced by a Milli-RO 15 plus Milli-Q purification system (Millipore, Bedford, MA, USA).

Equipment

The vacuum filtration assembly was supplied by the Millipore corporation Bedford, MA, USA; the solid phase extraction C₁₈ cartridge by Supelco (Bellfonte, PA, USA); the solid phase extraction vacuum manifold by VisiprepTM DL 9 Supelco; Bellfont, PA, USA; and HPLC system by Shimadzu (LC10A, detector UV, SPD-M10AV, Japan).

Solid Phase Extraction (SPE) of aspartame from *Kalakand*

The sample preparation procedure (Fig. 2) used for isolation of aspartame from *kalakand* was essentially based upon the method of BS EN:12856 (1999) used for semisolid and solid products. A blank experiment was also performed simultaneously.

Fig. 1 Flowchart for manufacture of *kalakand*

HPLC analysis

High-performance liquid chromatography analysis of reference standards of aspartame, degradation products and sample isolates from *kalakand* were performed under the following set of standardized conditions:

Column stationary phase	: Shimpak C ₁₈ , S-5 µm, 120A, 250×4.6 mm ID	
Phase	: Reverse phase	
UV detector wavelength	: 200 nm	
Mobile Phase (A)	: 0.02 M phosphate buffer, pH 5.0: acetonitrile	(97:3)
Mobile Phase (B)	: 0.02 M phosphate buffer, pH 3.5: acetonitrile	(80:20)
Flow rate	: 1 ml/min	
Run time	: 30 min.	
Maximum pressure	: 400 kgmf	

Binary gradient programming

Time (min)	B concentration (%)	Actual pressure observed (Kgmf)
0.1	0	80
8	0	80
13	100	160
25	100	160
27	0	80
30	STOP	80

Standard solutions of aspartame and degradation products

Ten milligram each of aspartame and its degradation products were dissolved in a 10 mL mixture of mobile phases A and B (1:1) to give stock standard solutions of concentration 1 mg/mL were pipetted out into separate 10 mL volumetric flasks and the volume made up to the mark with mixture of mobile phases A and B (1:1), to give standard solution of concentration 10 ng/µL. Also, 100 µL from each of the standard solutions (1 mg/mL) were pipetted out into a 10 mL volumetric flask and volume made up to the mark with mixture of mobile phases A and B (1:1), to give a mixture of aspartame and degradation products in standard solutions of concentration 10 ng/µL. A 5-point calibration curves were plotted for aspartame and its degradation products. Curves were prepared representing 50, 100, 200 and 250 ng concentration and their corresponding peak areas. The correlation coefficient of 0.99 for aspartame as well as its degradation products showed the linearity of the system. Recovery procedures of aspartame and

Fig. 2 Flowchart for isolation of aspartame from *Kalakand*

its degradation products were performed at 800 and 625 ppm, respectively in *kalakand*.

Sample preparation and HPLC analysis

The sample preparation was standardized to 1.75 g of *kalakand* to ensure completely fat free extraction through C₁₈ cartridges. The amounts of Carrez solutions 1 and 2 required for the precipitation of proteins in *kalakand* was standardized as 6 mL each. Elution was done with 10 mL of mobile phase B for efficient recovery of aspartame and its degradation products.

Storage and analysis of *kalakand*

Control and artificially sweetened samples of *kalakand* (with best selected sweetener level) were stored at refrigerated temperature (6–8 °C) and samples were analysed at 0, 3 and 7 days of storage for titratable acidity and pH IS: SP-18 Part XI (1981), microbiological examination IS: SP-18 Part I (1980) using Plate Count Agar; incubation of the plates was carried out at 37 °C for 48 h.

Sensory evaluation

The samples of *kalakand* were evaluated for the sweetness, colour and appearance, body and texture and overall acceptability by a panel of 8 judges from faculty and students of Dairy Chemistry Division, National Dairy Research Institute,

Karnal using a 9-point hedonic scale score card (Amerine et al. 1965).

Colour measurement

The colour of *kalakand* was measured by using a Colourflex (Hunter lab, Reston, VA, USA) with universal software 9 version 4.10. The light source was xenon flash lamp. Sample was filled upto 2/3rd of the capacity in a cylindrical glass bowl (2.5 cm height and 5 cm diameter) and readings were noted down. Data was received through the software in terms of L* [lightness, range from zero (black) to 100 (white)], a* [redness, range from +60 (red) to -60 (green)] and b* [yellowness, range from +60 (yellow) to -60 (blue)] values of the international colour system.

Statistical analysis

In all experiments, one-way/two-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 groups as described by Snedcor and Cochran (1989). Three replications of each experiment were analyzed for statistical analysis.

Results and discussion

Optimization of aspartame levels in *kalakand*

Sensory evaluation revealed that aspartame when used at the levels of 0.055, 0.060 and 0.070 % in *kalakand* (Table 1) resulted in lower sensory scores than control. The level of 0.065 % in *kalakand* scored highest in terms of sweetness perception and resembled control. Colour and appearance and body and texture scores were significantly lower in aspartame sweetened *kalakand* as compared to control. However, overall acceptability of aspartame sweetened *kalakand* was lower ($P < 0.05$) than the corresponding controls. This is because sugar (sucrose) not only contributes towards the sweetness but also helps in development of desirable flavor, body and texture and

characteristic colour through maillard browning and caramelisation. Hence, on the basis of sensory evaluation, out of the above three levels, the level of 0.065 % in *kalakand* was finally selected for the manufacture.

Analysis of *kalakand* during storage

Sensory profile

Sensory scores during storage (Table 2) revealed that aspartame-sweetened *kalakand* resembled the control in retaining the sensory profile (sweetness, colour and appearance, body and texture, and overall acceptability) at all periods of storage. However, aspartame sweetened *kalakand* ranked lower ($P < 0.05$) than control. The scores in control *kalakand* could be attributed to sucrose. On other hand, addition of aspartame resulted in insufficient water-binding capacity due to lower quantity of addition leading to product with a slightly lower score.

Titrateable acidity and pH

There was no apparent difference in initial titrateable acidity of the control and aspartame-sweetened *kalakand* (Table 2). It is evident that there was a significant ($P < 0.05$) increase in acidity of control as well as aspartame sweetened *kalakand* during storage. There was a significant difference ($P < 0.05$) in titrateable acidity between control and *Kalakand* sweetened with aspartame. This difference in titrateable acidity may be due to the slight preservative effect of sucrose, which led to the retarded microbial growth. The pH of aspartame-sweetened *kalakand* decreased ($P > 0.05$) during storage. The pH of control and aspartame sweetened *kalakand* were similar initially as well as at different periods of storage.

Colour measurement

Changes in lightness L^* and redness a^* values was not significant ($P > 0.05$) during storage for both control and artificially sweetened *kalakand* (Table 2). The slight change in these colour parameters was due to the Maillard type of browning reaction in both control and aspartame containing

Table 1 Sensory quality of *kalakand* containing different levels of aspartame

Characteristics	Control	Aspartame levels			
		0.055 % 550 ppm	0.060 % 600 ppm	0.065 % 650 ppm	0.070 % 700 ppm
Sweetness	8.0±0.00 ^a	6.7±0.34 ^b	6.7±0.25 ^b	7.7±0.16 ^a	6.2±0.37 ^b
Body & texture	7.6±0.17 ^a	6.3±0.25 ^b	6.4±0.22 ^b	6.4±0.31 ^b	6.5±0.18 ^b
Colour & appearance	7.6±0.18 ^a	6.9±0.14 ^b	7.0±0.18 ^b	7.0±0.25 ^b	7.0±0.18 ^b
Overall acceptability	7.7±0.16 ^a	6.1±0.22 ^b	6.2±0.25 ^b	7.1±0.25 ^c	6.8±0.18 ^c

Means in each row with different superscripts (a, b, c) were significantly different (LSD test, $P < 0.05$) from each other. Data are presented as means ± SD ($n = 8$)

Table 2 Changes in quality of aspartame (650 ppm) sweetened *kalakand* during storage at 6–8 °C

Particulars of <i>kalakand</i>	Storage (Days)		
	0	3	7
Sensory quality			
Sweetness			
Control	8.1±0.12 ^{Aa}	8.1±0.12 ^{Aa}	8.0±0.12 ^{Aa}
Aspartame	7.6±0.16 ^{Ba}	7.6±0.16 ^{Ba}	7.5±0.17 ^{Ba}
Color and appearance			
Control	8.0±0.18 ^{Aa}	7.8±0.12 ^{Aa}	7.8±0.20 ^{Aa}
Aspartame	7.6±0.18 ^{Ba}	7.5±0.18 ^{Ba}	7.2±0.31 ^{Ba}
Body and texture			
Control	7.8±0.12 ^{Aa}	7.8±0.12 ^{Aa}	8.1±0.12 ^{Aa}
Aspartame	6.7±0.36 ^{Ba}	6.8±0.22 ^{Ba}	6.5±0.17 ^{Ba}
Overall acceptability			
Control	8.0±0.26 ^{Aa}	8.0±0.26 ^{Aa}	8.0±0.17 ^{Aa}
Aspartame	7.1±0.27 ^{Ba}	7.0±0.27 ^{Ba}	6.6±0.48 ^{Ba}
Physicochemical			
Titratable acidity (%LA)			
Control	0.35±0.01 ^{Aa}	0.37±0.00 ^{Ab}	0.40±0.00 ^{Ac}
Aspartame	0.34±0.00 ^{Aa}	0.41±0.00 ^{Bb}	0.43±0.00 ^{Bc}
pH			
Control	6.37±0.00 ^{Aa}	6.35±0.01 ^{Aa}	6.29±0.00 ^{Ab}
Aspartame	6.35±0.00 ^{Aa}	6.32±0.03 ^{Ab}	6.25±0.00 ^{Bc}
L (light ness)			
Control	65.7±0.30 ^{Aa}	64.7±0.30 ^{Aa}	63.2±0.41 ^{Ab}
Aspartame	66.5±0.16 ^{Aa}	65.6±0.05 ^{Aa}	65.3±0.12 ^{Ba}
A (red ness)			
Control	6.2±0.09 ^{Aa}	7.2±0.18 ^{Ab}	7.2±0.05 ^{Ab}
Aspartame	5.9±0.22 ^{Aa}	6.3±0.10 ^{Aa}	6.9±0.11 ^{Ab}
B (yellow ness)			
Control	31.8±0.10 ^{Aa}	30.9±0.15 ^{Aa}	30.7±0.23 ^{Aa}
Aspartame	30.5±0.11 ^{Aa}	31.2±0.13 ^{Aa}	30.5±0.10 ^{Aa}
Microbiological quality			
Total plate count (log CFU/ml)			
Control	1.6×10 ³ ±0.32 ^{Aa}	3.6×10 ³ ±0.92 ^{Ab}	5.6×10 ³ ±0.26 ^{Ac}
Aspartame	2.0×10 ³ ±0.28 ^{Aa}	5.0×10 ³ ±0.69 ^{Bb}	10.3×10 ³ ±1.04 ^{Bc}

Means in each row with different superscripts (a, b, c) were significantly different (LSD test, $P < 0.05$) from each other. Means in a column with different superscripts (A, B) were significantly different (LSD test, $P < 0.05$) from each other. Data are presented as means ± SD (n=8)

samples. It was also noted that the lightness was less in control samples than aspartame sweetened *kalakand* during the entire

period of storage, which might be due to slight caramelization in presence of sucrose. Results obtained were in accordance

Fig. 3 HPLC chromatogram of aspartame and its degradation products standards

Table 3 Regression equation and correlation coefficient of aspartame and its degradation products

Product	Regression equation	R ² correlation coefficient
Aspartame	Y=1903.2X–2026.6	0.9992
Diketopiperazine	Y=3978.7X–2951.8	0.9998
L-Phenylalanine	Y=2740.6X–1130.5	0.9987

with the results of Gothwal and Bhavdasan (1991), who reported an increase in browning as a result of storage in *khoa*.

Microbiological examination

Total plate counts were higher ($P<0.05$) in aspartame sweetened product than their corresponding control throughout the storage period (Table 2) as there was no preservation/protection effect of these sweeteners unlike sugar, which ultimately led to higher microbial counts. There was an

increase ($P<0.05$) in the total plate counts in both the control *kalakand* and the aspartame sweetened products during the storage period.

Stability of aspartame

Mazur (1974) has discussed the degradation of aspartame into its degradation products like diketopiperazine, L-phenylalanine, Aspartic acid, Methanol etc. with structure. Aspartame, diketopiperazine and L-phenylalanine gave $\lambda_{\max}$ at 200 nm. Figure 3 represents the HPLC chromatogram of these three components at 200 nm under the standardized analytical conditions. The degradation products are eluted by mobile phase A and the main sweetener aspartame by mobile phase B.

The regression equations and correlations coefficient obtained are depicted in the Table 3. The correlation coefficient of 0.99 for aspartame as well as degradation products showed linearity of the system. The detection limits were in the range

Fig. 4 HPLC chromatograms of sample isolates of aspartame sweetened *kalakand* during storage

of 10–30 ng. Lawrence and Charbonneau (1988) also reported similar observations for aspartame. However, no information is available in the literature regarding the detection limits of these degradation products of the sweetener. Samples of *kalakand* sweetened with optimized aspartame levels were isolated and analyzed over HPLC under the standardized set of conditions. The recovery of the method was 95–98 %.

Stability of aspartame in kalakand during storage

Aspartame in *kalakand* maintained level of 638.30 ppm, 633.94 ppm and 625.77 ppm on day 0, 3 and 7 of storage with recovery of 98.20, 97.53 and 96.27 % respectively. It is evident from the data that the aspartame levels remained unchanged even upto 7 days of storage in *kalakand*, establishing thereby the stability of aspartame during storage.

HPLC chromatograms (Fig. 4) obtained on 0, 3rd and 7th days of storage for *kalakand* also supported this observation as there was no peak other than aspartame in these chromatograms. This established that aspartame was not degraded during storage in *kalakand* samples under investigation. This might be due to pH of *kalakand* (6.2) and its low moisture content ($a_w=0.764$). Bell and Labuza (1994) observed that decreasing the pH from 6.7 to 6.4 doubled the stability of aspartame in the dairy beverages sweetened with aspartame. Arman and Temiz (1988) evaluated aspartame degradation in reduced moisture toothpaste at pH 6.05. They found a half life of 114 days at 40 °C for a system with water activity (a_w) determined experimentally to be 0.76. Aspartame is unstable if subjected to prolonged heating and therefore cannot be used in baking or cooking unless added at the end of the cooking process (Kroger et al. 2006), as has been done in the preparation of sweetened *kalakand* in the present study.

Conclusion

Sensory profile during storage studies at 6–8 °C revealed that aspartame-sweetened *kalakand* resembled the control *kalakand* in retaining sweetness. The optimum level of aspartame was found to be 0.065 % in *kalakand* on the basis of sensory evaluation. However, aspartame-sweetened *kalakand* ranked lower than the control in sensory, acidity and microbial load. A solid phase extraction (SPE) method was standardized for the isolation of aspartame from *kalakand* followed by HPLC. High-performance liquid chromatography analytical

conditions were standardized for the separation of aspartame and its degradation product diketopiperazine and L-phenylalanine over a C_{18} column at UV 200 nm. Recovery of the method was 90–97 %. The results of the present investigation have established the successful use of aspartame in the preparation of the indigenous dairy product *kalakand*.

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