Shelf-life and microbiological safety studies of refrigerated *petha* sweet

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Abstract This study was conducted at Indian Institute of Vegetable Research, Varanasi (I.I.V.R.), Uttar Pradesh from 2006 to 2009 for evaluating comparative physico-chemical, microbiological and sensory attributes of *crystallized* and *Kashi petha* at refrigerated storage (4 ± 1 °C) condition up to 90 days. The *petha* were manufactured using 2 varieties of ash gourd developed at I.I.V.R. i.e.; Kashi Dhawal and Kashi Ujwal, selected based on different parameters suitable for *petha* sweet preparation. The used packaging materials for storage studies were laminated pouch, low density polyethylene, high density polyethylene. The physiochemical analysis and sensory evaluation were performed at 15 days interval upto 90 days for storage study in both *Kashi Petha* and *crystallized petha*, while microbial observation was recorded up to 90 days in *crystallized petha* and up to 50 days *Kashi Petha*. Physico-chemical analysis exhibited the continuous increase in moisture (25.79–26.80 % and 31.68–32.63 %) and titratable acidity (0.022–0.029 %) and (0.022–0.030 %) and reduction in ascorbic acid content (5.50–5.00 mg/100 g and 5.12–4.84 mg/100 g), respectively in *crystallized* and *Kashi petha*. The increasing trend in microbial population was more pronounced in *Kashi petha* in comparison to *crystallized petha*. The spoilage bacteria included *Bacillus* spp., *Staphylococcus* spp. and lactic acid bacteria while *Aspergillus*, *Penicillium* and *Saccharomyces* were observed among fungal contaminants. The bacteria, yeast and mold were identified on the basis of cultural, morphological and biochemical characterization with respect to storage period.

On the basis of the overall findings of comparative physico-chemical, microbiological and sensory evaluation, 30 and 50 days of storage was considered as safe for the consumption of *Kashi* and *crystallized petha*, respectively under refrigerated environment (4 ± 1 °C).

Keywords Ash gourd · *crystallized petha* · *Kashi petha* · Shelf-life · Microbiological safety

Introduction

Ash gourd (*Benincasa hispida*; syn. white gourd, wax gourd, white pumpkin, winter melon, ash pumpkin, hairy melon and Chinese preserving melon) is one of the important cucurbit vegetables of India. Fruits of ash gourd have long shelf-life and bright scope of value addition. It contains substantial amount of moisture (96.4 %), protein (467.67 mg/100 g), fat (102.63 mg/100 g), vitamin C (10.76 mg/100 g), dietary fiber (0.8 g/100 g) and carbohydrates 2.60 % (Pandey et al. 2009). Ash gourd also possesses several medicinal properties. It is useful for the treatment of epilepsy (Ramesh et al. 1989), peptic ulcer (Grover et al. 2001) and urinary infection (Nayar and More 1998). The fruits are consumed as baked, fried, boiled, pickled, candied and preserved (Robinson and Decker-Walters 1999).

The fruits of ash gourd in India are commercially utilized for manufacturing candy and a variety of sweet delicacy called *petha* in northern India and as culinary vegetable in southern and eastern part of country. Agra city of Uttar Pradesh (U.P.) of India has become a renowned business centre for production and processing of *petha* sweet. In India, *petha* is manufactured at cottage scale under unhygienic conditions leads to the rapid deterioration and become unacceptable to consumers in a limited time period. Along with, the confectioners do not follow the standard procedure for manufacturing. The deleterious changes are

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primarily due to objectionable off-flavor induced by microbial activities leads to short shelf-life of *petha* sweet resulting in reduced commercial production and distribution.

Extending the shelf-life of *petha* sweet by standardizing the manufacturing protocol at commercial scale is one of the major concerns for the processing industries. The shelf-life depends on several factors, particularly initial microbial population, storage temperature, packaging materials and the gaseous environment around the product. Microbiological safety of ash gourd products is a complex fundamental issue to manage the safety in production and processing industries. Therefore, any technique that could control these factors may be the key for the extension of the shelf-life of ash gourd products. In the present study, a comparative analysis on physico-chemical, microbiological and sensory quality was carried out up to 90 days of storage at 4 ± 1 °C for *crystallized* and *Kashi petha* prepared from IVAG-90 (*Kashi Ujwal*) and IVAG-502 (*Kashi Dhawal*) variety of ash gourd to determine the effective days of storage and overall acceptability.

Materials and methods

Selection of suitable ash gourd variety

Fully matured fruits of two released and notified varieties of ash gourd i.e.; ‘IVAG-90’ and ‘IVAG-502’ developed at Indian Institute of Vegetable Research (I.I.V.R.), Varanasi (Kalloo et al. 2006) were obtained for manufacturing of *petha*. The selection of suitable varieties was on the basis of optimum horticultural traits and shelf life, texture and physico-chemical properties of fruit (Pandey et al. 2009).

Petha manufacturing protocol

The manufacturing protocol of *crystallized* and *Kashi petha* was optimized by applying an experimental model by response surface methodology (RSM) using 03 independent variables such as lime water concentration (05–15 %), treatment time (10–30 min) and sugar concentration (50–90 %). The *crystallized petha* was prepared as preserved candy whereas *Kashi petha* was preserved in sugar syrup with 0.20 % citric acid. The statistical significance of the terms was examined by analysis of variance (data not shown). Response surface plots were generated with the same software and optimization of process parameters was performed. The model was assessed to evaluate the various responses (data not shown).

Storage studies

Physico-chemical, microbial and sensory attributes of *crystallized* and *Kashi petha* packed in laminated (150 gauge) pouches were monitored at regular interval of 15 days for a

period of 90 days at 4 ± 1 °C. In a separate study of suitable packaging material for shelf-life extension of *petha*, laminated pouch was observed best packaging material in a comparative study of LDPE (low density polyethylene), HDPE (high density polyethylene), polypropylene and laminated pouches as far as the physico-chemical, microbial and sensory attributes was concerned. Data has not been given.

Physico-chemical analysis

The physico-chemical parameters such as *pH*, titratable acidity, moisture content and ascorbic acid content were analyzed initially and at the interval of 15, 30, 45, 60, 75 and 90 days of refrigerated storage at 4 ± 1 °C using standard analytical method (Ranganna 1997).

Microbial observation

Bacterial, yeast and mould populations in *crystallized* and *Kashi petha* initially and at the interval of 15, 30, 45, 60, 75 and 90 days at 4 ± 1 °C were observed as viable count (log CFU/g) by serial dilution-agar plate technique (Aneja 2001). The cultural, morphological and biochemical characterization for the identification of bacteria, yeast and mould were carried out by Aneja (2001).

Sensory evaluation

A panel of 10 trained judges was set up to evaluate *crystallized* and *Kashi petha* for overall acceptability with regards to flavor, color and appearance and body and texture on 9-point hedonic scale as per the method (Amerine et al. 1965).

Statistical analysis

Analysis of variance and critical difference for triplicate data of each observation ($n=3$) was performed after logarithmic transformation of all values (Snedecor and Cochran 1989).

Results and discussion

Petha manufacturing protocol

The experimental data used for optimization study were obtained using a central composite rotatable design. Overall acceptability score was obtained after treatment with 5 % lime water concentration for both crystallized and Kashi Petha, 120 min lime water treatment time for both crystallized and Kashi Petha and 65 % sugar concentration for Kashi Petha and 75 % in crystallised petha (data not shown). This protocol has been optimized to manufacture the petha for microbial evaluation and storage study.

Physico-chemical evaluation

Moisture content

The changes in moisture content of *crystallized* and *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during refrigerated storage has been statistically analyzed in Table 1a and b. The increasing trend in moisture content was observed from the initial 25.7–32.3 % to final 26.2–32.6 % in *crystallized* and *Kashi petha*, respectively. *Kashi petha* exhibited more moisture content (32.2 and 32.6 % in 'IVAG-502' and 'IVAG-90', respectively) than the *crystallized petha* (26.2 and 26.8 % in 'IVAG-502' and 'IVAG-90', respectively) because the *crystallized petha* was prepared as dried candy and *Kashi petha* was preserved in sugar syrup thus absorbed more moisture content during storage as compared to *crystallized petha*. The increase in moisture content in subsequent days was not very pronounced in the early days of storage. Saxena et al. (1996) has also reported the increasing trend of moisture content in stored pinni (an Indian traditional sweet) under ambient condition.

pH

The changes in pH of refrigerated *crystallized* and *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502'

during storage has been statistically analyzed in Table 1a and b. The pH of *crystallized* and *Kashi petha* was decreased up to 5.9–5.4 from the initial pH 6.89–7.0 with increasing period of storage at 4 ± 1 °C. The pH was decreasing more in *Kashi petha* (6.8–5.4) as compared to *crystallized petha* (6.9–5.9). This drop in pH might be explained due to occurrence of such micro flora in the *petha* which utilized the sugar as fermentable substrate resulting in the production of acids (Osuntogun et al. 2004). The comparative more decreased trend in pH of *Kashi petha* was supposed to be due to more moisture content of sugar syrup which resulted in the rapid growth of microbial population and utilization of fermentable sugars in to acids. Similar trend of decrease in pH was also reported during storage of carrot pickle (Chawla et al. 2005).

Titratable acidity

The changes in the titratable acidity of *crystallized* and *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during refrigerated storage has been statistically analyzed in Table 1a and b. The titratable acidity in *crystallized petha* was increased up to 0.027–0.029 % from the initial acidity (0.022–0.023 %) with decreasing trend of pH. Similar increasing trend for titratable acidity during storage was also observed in *Kashi petha*. The increase in the

Table 1 Physico-chemical quality parameters of (a) *Crystallized petha* and (b) *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during refrigerated storage (4 ± 1 °C)

Interval days	pH		Titratable acidity (% citric acid)		Moisture content (%)		Ascorbic acid (mg/100 g)	
	IVAG-90	IVAG-502	IVAG-90	IVAG-502	IVAG-90	IVAG-502	IVAG-90	IVAG-502
(a) <i>Crystallized petha</i>								
0	7.0 ^{ay}	6.9 ^a	0.022 ^f	0.023 ^g	26.6 ^c	25.7 ^g	5.4 ^a	5.5 ^a
15	7.0 ^a	6.8 ^a	0.022 ^e	0.023 ^f	26.6 ^d	25.8 ^f	5.3 ^{ab}	5.4 ^b
30	6.8 ^b	6.7 ^b	0.023 ^d	0.024 ^e	26.6 ^c	25.9 ^e	5.3 ^b	5.4 ^c
45	6.7 ^b	6.6 ^c	0.024 ^c	0.026 ^d	26.6 ^c	25.9 ^d	5.1 ^c	5.2 ^d
60	6.5 ^c	6.4 ^d	0.026 ^b	0.027 ^c	26.7 ^c	26.0 ^c	5.1 ^d	5.1 ^e
75	6.2 ^d	6.2 ^e	0.026 ^b	0.028 ^b	26.7 ^b	26.1 ^b	5.0 ^d	5.1 ^f
90	6.0 ^e	5.9 ^f	0.027 ^a	0.029 ^a	26.8 ^a	26.2 ^a	5.0 ^e	5.0 ^g
SD (±)	0.38	0.35	0.002	0.002	0.078	0.17	0.16	0.19
CD at 0.05 (n=3)	0.141	0.029	2.80	1.979	0.298	0.029	0.787	0.022
(b) <i>Kashi petha</i>								
0	7.0 ^{ay}	6.8 ^a	0.022 ^f	0.023 ^g	32.3 ^e	31.6 ^c	5.1 ^a	5.0 ^a
15	7.0 ^a	6.8 ^a	0.022 ^f	0.024 ^f	32.3 ^e	31.7 ^{bc}	5.0 ^{ab}	5.0 ^b
30	6.9 ^b	6.7 ^b	0.024 ^e	0.025 ^e	32.3 ^e	31.8 ^{abc}	5.0 ^{abc}	4.9 ^b
45	6.8 ^c	6.5 ^c	0.025 ^d	0.026 ^d	32.3 ^d	31.9 ^{abc}	5.0 ^{bcd}	4.9 ^b
60	6.2 ^d	6.1 ^d	0.026 ^c	0.027 ^c	32.4 ^c	32.0 ^{abc}	5.0 ^{cde}	4.9 ^c
75	6.0 ^e	5.9 ^e	0.027 ^b	0.028 ^b	32.5 ^b	32.1 ^{ab}	4.9 ^{de}	4.8 ^d
90	5.4 ^f	5.7 ^f	0.030 ^a	0.030 ^a	32.6 ^a	32.2 ^a	4.9 ^e	4.8 ^d
SD (±)	0.61	0.45	0.002	0.002	0.12	0.22	0.07	0.08
CD at 0.05 (n=3)	0.101	0.028	2.853	2.486	0.032	0.389	0.072	0.043

* Data are the replication of 3 years (2006–2009)

y- Values within column followed by the different letters are significantly different at 5 % level according to Duncan's multiple range test
CD Critical difference, SD Standard deviation

Table 2 Changes in microbiological population in (a) *Crystallized petha* and (b) *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during refrigerated storage (4 ± 1 °C)

	(a) <i>Crystallized petha</i>						
	0	5.0d ^y	4.8d	3.6c	3.5d	3.3d	3.3c
	30	5.0 d	4.8d	3.6c	3.5d	3.3d	3.3c
	50	5.3c	5.8c	5.0b	5.2c	4.1c	4.2b
	75	6.7b	6.3b	5.8a	5.3b	4.9b	4.9a
	90	7.8a	6.8a	5.9a	5.6 a	5.4a	4.9a
	SD (±)	1.2	0.89	1.1	1.05	0.93	0.79
*Data are the replication of 3 years (2006–2009)	CD at 0.05 (<i>n</i> =3)	0.13	0.02	0.16	0.076	0.14	0.09
	(b) <i>Kashi petha</i>						
y- Values within column followed by the different letters are significantly different at 5 % level according to Duncan’s multiple range test	0	3.7b ^y	3.64b	5.0b	4.9b	3.2b	3.3b
	30	3.7b	3.64b	5.0b	4.9b	3.2b	3.3b
	50	7.8a	7.2a	6.9a	7.2a	4.4a	4.43a
	SD (±)	2.3	2.0	1.1	1.3	0.68	0.65
CD Critical difference, SD Standard deviation	CD at 0.05 (<i>n</i> =3)	0.04	0.01	0.02	0.07	0.02	0.23

*Data are the replication of 3 years (2006–2009)

y- Values within column followed by the different letters are significantly different at 5 % level according to Duncan's multiple range test

CD Critical difference, SD Standard deviation

titratable acidity during storage directly correlated with the drop of pH due to the production of organic acids during the fermentation of carbohydrates in *petha* (Osuntogun et al. 2004). The titratable acidity increase was more in *Kashi petha* (0.022–0.030 %) than *crystallized petha* (0.022–0.029 %) due to more moisture content of sugar syrup which resulted in the rapid growth of microbial population and utilization of fermentable sugars in to acids. Increasing trend in acidity during a period of time of storage of carrot pickle (Chawla et al. 2005) and kinnow juice was also reported (Singh et al. 2009).

Ascorbic acid

The changes in ascorbic acid content of *crystallized* and *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during refrigerated storage has been statistically analyzed and presented in Table 1a and b. The decrease in ascorbic acid content was obtained up to 5.0 mg/100 g from the initial 5.4–5.5 mg/100 g with increasing period of storage in *crystallized petha*. In *Kashi petha*, decreasing trend was statistically significant from initial days to advancement of storage period in both the varieties. Similar decreasing trend in ascorbic acid content was also reported during advancement of storage period in some products such as mango-pineapple spiced beverages, lime aonla spiced beverages and some juices of tomato, amla, carrot, carrot-spinach, carrot-pineapple, and carrot-beetroot (Agarwal et al. 1995; Dhaliwal and Hira 2001, 2004; Dekka et al. 2004, 2005). *Kashi petha* had exhibited the more decreased ascorbic acid content from 4.8 to 4.9 mg/100 g in IVAG-90 and

IVAG-502 than the *crystallized* (5.0 mg/100 g) *petha* in both the variety. This decrease in ascorbic acid content might be due to the more oxidation of ascorbic acid in refrigerated environment.

Microbiological observation

The changes in microbiological population (bacteria, yeast and moulds) in fresh and stored samples of *crystallized* and *Kashi petha* has been statistically analyzed and presented in

Table 3 Sensory analysis of *Crystallized petha* and *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during refrigerated storage (4 ± 1 °C)

Interval days	Overall acceptability score			
	<i>Crystallized petha</i>		<i>Kashi petha</i>	
	IVAG-90	IVAG-502	IVAG-90	IVAG-502
0	7.5	6.9	7.0	7.2
30	6.5	6.0	6.2	6.0
50	6.1	6.0	5.0	5.8
75	5.0	5.1	3.6	4.2
90	4.6	5.0	3.3	3.2
SD ($\pm$)	1.1	0.76	1.6	1.6
CD at 0.05 ($n=3$)	0.31	0.36	0.23	0.21

*Data are the replication of 3 years (2006–2009)

y- Values within column followed by the different letters are significantly different at 5 % level according to Duncan's multiple range test
CD Critical difference, SD Standard deviation

Table 2a and b, respectively. There had been increasing trend in bacterial (4.8–7.8 log CFU/g), yeast (3.3–5.4 log CFU/g) and moulds population (3.4–5.9 log CFU/g) in *crystallised petha*. In *Kashi petha*, significant increase in bacterial growth was found at last count as compare to initial stage, whereas the growth of yeast and mould was more but non-significant. The increasing trend in microbial count was also reported by Chawla and Ranote (2009). The growth of microbial population at low temperature retarded the spoilage without preventing the multiplication of microbial population. Refrigerated *petha* was therefore subjected to spoilage by bacteria, yeasts and moulds (Osuntogun and Aboaba 2004). Among bacterial population, *Bacillus* spp., *Staphylococcus* spp. and lactic acid bacteria such as *Lactobacillus* spp. were observed. However, *Aspergillus* and *Penicillium* among moulds and *Saccharomyces* were observed in yeast population. The similar observation was also reported by Rhodes and Fletcher (1966) in sugary and carbohydrate rich foods. The occurrence of *Staphylococcus* in *petha* might be explained by unhygienic processing of the product leading to the contamination by natural micro-flora of the raw materials and the utensils (Osuntogun and Aboaba 2004).

Bacterial population (log CFU/g) ranged from 4.8 to 7.8, yeast population from 3.3 to 5.4 and moulds population from 3.5 to 5.9 in *crystallized petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during storage at 4 ± 1 °C. However, bacterial population ranged from 3.6 to 7.8 log CFU/g, yeast population from 3.2 to 4.4 log CFU/g and moulds population from 4.9 to 7.2 log CFU/g in *Kashi petha* of ash gourd variety 'IVAG-90' and 'IVAG-502' during 50 days of storage at 4 ± 1 °C. Bacterial population was more in both types of *petha* than the yeast and moulds. The population of bacteria, yeast and moulds (log CFU/g) was more pronounced in *Kashi petha* than the *crystallized petha* at the time of 3rd observation during storage might be due to more moisture content which created the favorable environment for microbial growth and the fermentation of sugar resulting in the high acidity in *petha*. Manay and Swamy (1996) observed that higher sugar content with lower moisture do not favour microbial growth in food product. In such acidic product, *Bacillus* and *Lactobacillus* grow easily and produce organic acids resulting in the more titratable acidity of the product. Therefore, *Kashi petha* was considered as edible up to 40–45 days of storage at 4 ± 1 °C. However, *crystallized petha* had its natural taste and appearance up to 60–65 days of storage due to less moisture content and permissible range of microbial population in refrigerated environment (4 ± 1 °C). *Petha* has been considered a kind of ready to eat food in which all the ingredients are fully cooked during manufacturing and thus included in the category B according to the interpretive guides for ready to eat food published by the United Kingdom's Health Protection Agency and by Food Standards Australia New Zealand (2001). In this category of food the acceptable range of microbiological results has been reported $<10^7$ cfu/g or 7 log cfu/g.

Sensory evaluation

The changes in the sensory score on 9-point hedonic scale of refrigerated *crystallized* and *Kashi petha* of ash gourd genotype 'IVAG-90' and 'IVAG-502' during storage has been presented in Table 3. In *Kashi petha*, the overall acceptability score ranged from 7.2–3.2 and *crystallized petha* varied from 7.5–4.6 up to 90 days of refrigerated storage. Therefore, on the basis of the mean sensory scores of overall acceptability with regards to flavor, color and appearance and body and texture *crystallized petha* was considered edible up to 50 days and *Kashi petha* up to 30 days at 4 ± 1 °C. The products of fermentation such as acids, alcohols and diacetyls produced by bacteria, yeasts and moulds during the utilization of carbohydrates of *petha* for the fermentation processes affect the organoleptic attributes resulting in the undesirable changes in the products (Kannan and Thirumaran 2004).

Conclusion

This study indicated that *Kashi petha* and *crystallized petha* could be stored at refrigerated environment (4 ± 1 °C) for the period of maximum 30 and 50 days, respectively on the basis of acceptable physico-chemical, microbiological and sensory attributes.

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