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Development of value added green mango-mint-*Tulsi* squash by using honey as sweetner

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In present study, the value added squash was prepared by blending green mango-mint-*Tulsi* in various proportions and it was observed that of 85: 5: 10 (on weight basis) was most acceptable over other proportions. The squash from the blends were prepared with 25 per cent pulp, 50 per cent TSS, 1.0 per cent acidity, 1 g/l sodium benzoate and 2 per cent black salt. Various proportions (5-20%) of honey was also used to partially substitute sugars in the value added green mango-mint-*Tulsi* squash and it was observed that 20 per cent substitution improved maximum the organoleptic quality of the squash. The developed value added squash with or without honey was packed in transparent colourless glass bottles and stored at room temperature (35±5°C). During three months of storage period, total soluble solids (TSS), total sugars and reducing sugars increased significantly, whereas acidity and pH of the squashes did not change significantly. However, the ascorbic acid content, total carotenoids, total chlorophyll and total phenols of the squashes decreased significantly during storage period of three months. A non-significant change in the organoleptic scores for colour, appearance, flavour, taste, mouth feel and overall acceptability of green mango-mint-*Tulsi* squashes prepared with or without honey was observed during storage.

Key Words : Squash, Organoleptic quality, Honey, Chemical constituents, Sodium benzoate

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INTRODUCTION

Mango (*Mangifera indica* L.) belongs to family Anacardiaceae. Mango is rich in a variety of phyto-chemicals and nutrients. The fruit pulp is high in prebiotic dietary fibre, vitamin C, polyphenols and carotenoids. Mango contains essential vitamins and dietary minerals (Singh *et al.*, 2004). Mint (*Mentha viridis* L.) belongs to the family Lamiaceae and has common name '*Pudina*'. Mint leaves contain a number of vitamins and minerals, which are vital to maintain a healthy body. It is also said to relieve symptoms of indigestion, heartburn and irritable bowel syndrome by relaxing the muscles in and around the intestine, act as a powerful antioxidant, protecting the body against the formation of cancerous cells, very good cleanser for the blood and help in clearing up skin disorders such as acne (Aflatuni *et al.*, 2005). *Tulsi* (*Ocimum* sp.), also known as Basil, is an aromatic plant in the family Lamiaceae. *Tulsi's* extracts are used in *Ayurvedic* remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. Honey is a sweet viscous yellowish liquid with tempting flavours, which is elaborated by the honeybee from the nectar of plants. Beside antioxidants, acids (primarily gluconic acid), protein, minerals, flavonoids,

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vitamins and enzymes among are also found in honey (Wang *et al*., 2004). Blending of two or more fruit juices and their beverages with the addition of spice extracts/ drops as health drinks is thought to be convenient alternative for its utilization in order to have some value added fruit drinks, having high quality in respect of both sensory and nutritional aspects. (Joshi *et al*., 1993 and Gowda and Jalali, 1995).

Keeping in mind the nutritional and medicinal properties of green mango, mint and *Tulsi* and the flavour enhancing capacity of spices, the present research was undertaken with the following objectives:

Objectives:

– To prepare and evaluate the recipe for value added green mango-mint-*Tulsi* beverage.

– To study the shelf-life of the developed value added beverage.

METHODOLOGY

Extraction of pulp and paste making:

Green mango pulp:

Mango fruits were washed thoroughly with clean running water and cooked with 70 per cent of water in pressure cooker for 15 minutes. It was then brought to room temperature. Peel and stones were separated from the pulp with the help of a stainless steel knife. The extracted pulp was passed through fruit pulper to obtain fine pulp, packed in polyethylene bags and then stored under frozen condition (~ 20°C) to be used later.

***Tulsi* paste:**

Fresh leaves of *Tulsi* were washed under running tap water and then allowed to air dry. The paste was made by grinding the leaves in a grinder and by adding 100 ml water to 1kg of pulp to facilitate paste making. The paste was packed in polyethylene bags and stored at frozen condition (~ 20°C).

Mint paste:

Fresh tender twigs of mint with green leaves were washed thoroughly under running tap water and then allowed to air dry. The paste was made by grinding the leaves in a grinder and by adding 70 ml water to 1 kg pulp to facilitate paste making. The paste was packed in polyethylene bags and stored at frozen condition (~ 20°C).

Preparation of squash from green mango-mint-*Tulsi* proportions:

The extracted green mango pulp, *Tulsi* and mint pastes were blended in following proportions table.

Sr. No.	Green mango : Mint : *Tulsi*
1.	100:0:0
2.	95:5:0
3.	95:0:5
4.	90:5:5
5.	85:10:5
6.	85:5:10
7.	80:10:10

The procedure adopted for preparation of squash from the blends is presented in flow sheet (Fig. A). In brief, the total soluble solids (TSS) and acidity contents of blends were analyzed. Then requisite quantities of sugar syrup was prepared and calculated amount of citric acid was added to the blends to obtain final TSS and acidity as 50 per cent and 1 per cent, respectively. The squash prepared from various proportions was diluted 3 times and then organoleptically evaluated to obtain the best combination of the blend. The best combination of organoleptically evaluated squash blend, was reformulated by partially replacing sugar by 5, 10, 15 and 20 per cent

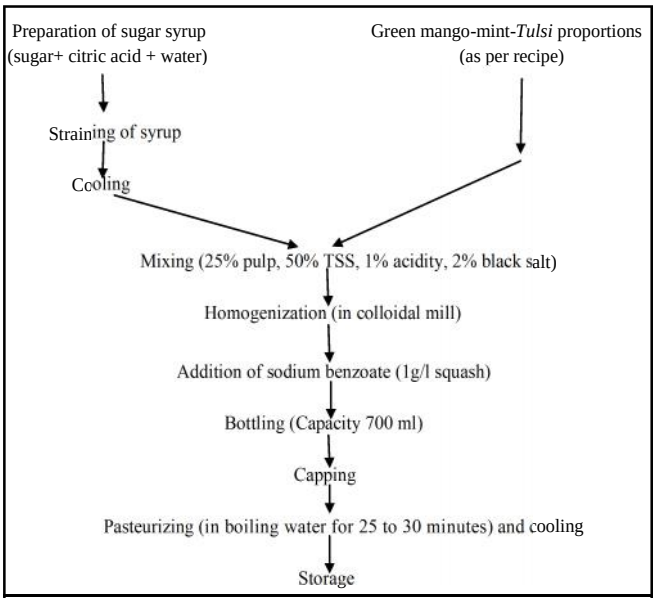

Fig. A: Flow sheet for preparation of green mango-mint-*Tulsi* squash

honey. The reformulated squash blend with various proportions of honey was diluted 3 times and then organoleptically analyzed to obtain best proportion of honey. To the best combinations was then added assorted spices black salt (2%). The best combination of above prepared squash blends with or without honey were separately bottled in 700 ml bottles, capped and stored at room temperature (35±5°C) for further analysis.

Chemical constituents:

Total soluble solids were estimated at ambient temperature by Abbe's Refractometer (0-95%) or by hand refractometer (0-32%) (Erma, Japan) and the values were expressed as per cent TSS. Total and reducing sugars were estimated by the method of Hulme and Narain (1931). Total acids were extracted in water and were estimated by titration method described by A.O.A.C. (2005). pH of product was estimated by pH meter (Model: CL 54 digital Toshniwal Instruments Mfg. Pvt. Ltd., India). The ascorbic acid was determined as given by A.O.A.C. (2005). Total carotenoids were determined according as per the method described by Rodriguez-Amaya (1999). Total chlorophyll was estimated as per the method of Arnon (1949). Phenols were estimated by the method of Amorium *et al*. (1997).

Organoleptic evaluation (9 point hedonic scale):

The squash was evaluated organoleptically by diluting it 3 times with water, at the regular monthly interval during three months storage. The organoleptic evaluation was done by a panel of ten semi trained judges following the hedonic rating scale as described by Ranganna (2003). The products were evaluated for colour and appearance, flavour, taste, mouth feel and overall acceptability.

Statistical analysis:

The data obtained in the present investigation were subjected to analysis of variance (ANOVA) techniques and thus, analyzed according to two factorial Completely Randomized Design (CRD). The critical difference value at 5 per cent level was used for making comparison among squashes with or without honey during storage period.

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Organoleptic evaluation:

On the basis of organoleptic evaluation (Table 1), the value added squash prepared by blending green mango:mint:tulsi in proportion of 85: 5: 10 (on weight basis) was found more acceptable (8.83) over other proportions. In case of various proportions (5-20%) of honey used to partially substitute sugars, substitution resulted in improvement in organoleptic sensory score for 5, 10, 15 and 20 per cent was 7.33, 7.33, 7.67 and 8.33, respectively (Table 2). From sensory evaluation the best treatment

Table 1 : Mean sensory evaluation scores of green mango-mint-*Tulsi* squash (during standardization)

Sr. No.	Green mango-mint-*Tulsi* proportions	Mean sensory scores
1.	100 : 0 : 0	7.33
2.	95 : 5 : 0	7.33
3.	95 : 0 : 5	7.33
4.	90 : 5 : 5	7.83
5.	85 : 10 : 5	8.00
6.	85 : 5 : 10	8.83
7.	80 : 10 : 10	7.67

Table 2: Mean sensory evaluation scores of partially honey substituted Green mango-mint-*Tulsi* squash (during standardization)

Sr. No.	Partial substitution of honey level (%)	Mean sensory scores
1.	5	7.33
2.	10	7.33
3.	15	7.67
4.	20	8.33

were selected for storage studies without honey the proportion of green mango: mint: *Tulsi* was 85:5:10 and for this ratio the partial substitution of honey for sugar was 20 per cent these two were further taken for storage studies.

Changes in chemical constituents of green mango-mint-*Tulsi* squash with or without honey during storage:

The chemical constituents changes in squashes without and with honey are presented in Table 3.

TSS:

The TSS of squashes prepared with or without honey was 50.3 per cent during preparation (0 day of storage). It was observed that, there was slight but significant increase in TSS of squash during storage, which increased from 50.3 per cent to 51.7 per cent in three months. This increase in total soluble solids content of the squash might be due to hydrolysis of polysaccharides into monosaccharides and oligosaccharides. In the present study, during storage, the total soluble solids content increased from 50.3 to 52.0 per cent and 50.3 to 51.3 per cent in the squash prepared without and with honey, respectively. However, no significant difference in TSS was observed between squash prepared with or without honey during entire storage period. The results of the present study are in agreement with findings of Majumdar *et al*. (2011) in bottleguard and basil leaves blended juice, Ismail *et al*. (2011) in whey based mango beverage and Deka *et al*. (2005) in mango-pineapple spiced beverages, where increased TSS during storage has been reported.

Total sugars and reducing sugars:

It was observed that there was a significant increase in total sugars of squashes upto two months of storage and it didn't change significantly during later storage. It was observed that there was slight but significant increase in reducing sugar of squashes during storage, which increased from 28.6 mg/100ml to 31.2 mg/100ml in three months. Throughout the storage period, significantly higher total sugars were observed in squash prepared without honey than with honey and reverse in case of reducing sugar.

The increase in reducing sugars might be due to inversion of non-reducing sugars into reducing sugars and hydrolysis of polysaccharides. Whereas, increase in total sugar might be due to the hydrolysis of polysaccharides like pectin, starch, etc. into simple sugars. The squash prepared with honey contained higher amount of reducing sugars than the squash prepared without honey. This might be due to the inherent content of 61.3 to 72.6 per cent total reducing sugars, 1.2 to 5.7 per cent sucrose and a small amount of non-reducing sugars in honey (Anupama *et al*., 2003). The reducing sugar content of squash prepared with honey increased from 30.5 to 32.8 mg/100ml during storage period. Similar results were found by Tandon *et al*. (2010) in case of mango pulp blending of different variety, Yadav *et al*. (2010) in whey-based banana herbal beverage with incorporation of *Mentha arvensis* extract, Deka *et al*. (2005) in mango-pineapple spiced beverages from '*Dashehari*' mango and '*Kew*' pineapple, Silva *et al*. (2008) in honey sweetened cashew apple juice, where increase in total sugar and reducing sugar contents was reported.

Acidity (%) and pH:

The acidity of the squashes prepared with or without honey was maintained at 1.1 per cent during preparation (0 day of storage) whereas pH of the squashes prepared with or without honey at 0-day of storage was 4.4. It was observed that the acidity and pH of squashes did not change significantly during storage, which remained constant (1.1%) even after three months of storage. Throughout the storage period, no significant difference in acidity and pH was observed between squashes prepared with or without honey. Yadav *et al*. (2010) observed a slight increase in acidity and a decrease in pH, during storage of whey-based banana herbal beverage incorporated with *Mentha arvensis* extract and attributed it to conversion of lactose to lactic acid, formation of organic acid by ascorbic acid, degradation of polyphenols and conversion of proteins to amino acids. Bandyopadhyay *et al*. (2008) reported increased acidity and decreased pH in carrot fortified milk product with beet and honey and attributed it to the formation of galacturonic acid by enzymatic breakdown of pectin in the raw material and high degree of acetylation of the pectin content.

Ascorbic acid (mg/100ml):

It was observed that there was significant and drastic reduction in ascorbic acid content of squashes during storage. The freshly prepared squash had 11.4 mg/100ml

of ascorbic acid which decreased to 3.4 mg/100ml during three months of storage. However, throughout the storage period, no significant differences in ascorbic acid content were observed between squashes prepared with or without honey.

In the present study, the reduction in ascorbic acid content might be due to the oxidation of ascorbic acid by oxygen present inside the bottle (head space) and that dissolved in the beverage. The results of present study are in agreement with the findings of Sood *et al*. (2010) who reported a decrease in ascorbic acid content of mango squash prepared by using cheese whey and soy-whey. They attributed the decrease to the unstable nature of the ascorbic acid and its breakdown to dehydro-ascorbic acid by the action of heat, air and light. Further increase in enzymatic and non-enzymatic oxidations reactions during storage period might have resulted in decrease in ascorbic acid content due to its participation in browning reaction.

Silva *et al*. (2008) reported that in honey sweetened cashew apple juice, the storage temperature and the incidence of light contributed to the reduction of vitamin C. A gradual loss in ascorbic acid of litchi beverages was observed by Karuna *et al*. (2005). Ismail *et al*. (2011) reported decrease in ascorbic acid content of whey based mango beverage due to degradation of ascorbic acid to carboxylic acid under high acidic condition.

Total phenols (mg/100ml):

It was observed that there was a significant and progressive decrease in total phenol content of squashes during storage. The maximum phenol content (18.2 mg/ 100 ml) was observed in freshly prepared squash without honey, which decreased to 12.3 mg/100 ml during three months of storage. However, throughout the storage period, significantly higher total phenols were observed in squashes prepared without honey than with honey. The average total phenol content of squash prepared without honey was 15.4 mg/100 ml, while with honey, it was 13.3 mg/100 ml.

The decrease could be attributed to oxidation of phenols. In general, the phenolic compounds are oxidized to O-semiquinone radicals or O-quinone molecules, which are highly reactive to give the brown products of high molecular weight. In the present investigation, throughout the storage period, the phenolic content was higher in squash prepared without honey than with honey. This may be probably due to the interactions between carbohydrates from honey and phenolic compounds in squash, making the hydroxyl groups of polyphenols unavailable for reaction with the FC reagent (Sharma *et al*., 2008). Similar results were obtained by Belscak *et al*. (2011) on the influence of honey addition in fruit tea infusions. Verma and Gehlot (2006) recorded a decrease in phenol content of bael beverages *viz.*, RTS drink, nectar and squash during storage period which might be due to their condensation into browning pigments.

Total carotenoids (mg/100ml):

It was observed that there was a significant and progressive decrease in total carotenoid content of squashes during storage. The maximum carotenoids (2.63 mg/100 ml) were observed in freshly prepared squash with honey and it decreased to 1.15 mg/100 ml during three months of storage. Significantly higher carotenoids were also observed in squash prepared with honey than without honey during entire storage period.

The retention of total carotenoids was more in case of squash prepared with honey as compared to without honey, probably due to inherent content of carotenoids in honey. Similar higher carotenoids in honey beverage were reported by Bandyopadhyay *et al*. (2008) in carrot fortified milk product prepared by using beet and honey.

In the present study, the decrease in total carotenoids during storage are in agreement with the findings of Karuna *et al*. (2005) in litchi beverages, where they reported that the decrease in total carotenoids during storage period was due to their sensitivity to oxygen, heat and light and their conversion into unusable forms. Nagpal and Rajyalakshmi (2009) also reported that total carotenoids decreased significantly in the bael and citrus fruit blends RTS beverages which might be due to oxidative breakdown and isomerization or enzymatic destruction of pigments. The decrease in carotenoids could also be due to its degradation by irreversible interaction of carotene with peroxyl radicals to produce a carotene radical. The carotene radical may react with other radicals such as lipid peroxyl radicals at low oxygen concentration and form non-radical products (Lee *et al*., 2004). Rodriguez-Amaya (1999) reported that losses of carotenoids during processing and storage might also occur due to physical removal, geometrical isomerization and enzymatic or non-enzymatic oxidation. Degradation of carotenoids occurs through isomerization of trans pro-

vitamin A to the cis-isomers which on further oxidation leads to the formation of low molecular mass compounds. Different factors like heat, light and acids promote isomerization of carotenoids (Dutta *et al*., 2005).

It was observed that there was slight but significant decrease in chlorophyll content of *squashes* during storage. The maximum chlorophyll content (5.11 mg/100 ml) was observed in freshly prepared squash with honey and it decreased to 2.01 mg/100 ml during three months of storage. However, throughout the storage period,

Table 3 : Changes in chemical constituents of green mango-mint-*Tulsi* squash with or without honey during storage

Chemical constituents	Storage / Treatment	0 day	30 days	60 days	90 days	Mean
% TSS	Without honey	50.3	52.0	51.7	52.0	51.5
	With honey	50.3	51.3	51.0	51.3	51.0
	Mean	50.3	51.7	51.3	51.7	
	C.D. (P=0.05)	Treatment = N.S.; Storage = 0.7 ; Treatment × Storage = 0.9				
Total sugars	Without honey	50.2	54.6	55.7	56.5	54.3
(mg/100ml)	With honey	49.9	54.8	55.1	55.4	53.8
	Mean	50.1	54.7	55.4	55.9	
	C.D. (P=0.05)	Treatment = 0.28; Storage = 0.34 ; Treatment × Storage = 0.55				
Reducing sugars	Without honey	26.7	27.9	28.6	29.5	28.2
(mg/100ml)	With honey	30.5	31.5	32.1	32.8	31.7
	Mean	28.6	29.7	30.4	31.2	
	C.D. (P=0.05)	Treatment = 0.50 ; Storage = 0.70 ; Treatment × Storage = 0.99				
Acidity (%)	Without honey	1.1	1.1	1.1	1.1	1.1
	With honey	1.1	1.1	1.1	1.1	1.1
	Mean	1.1	1.1	1.1	1.1	
	C.D. (P=0.05)	Treatment = N.S.; Storage = N.S. ; Treatment × Storage = N.S.				
pH	Without honey	4.4	4.5	4.5	4.5	4.5
	With honey	4.4	4.4	4.5	4.5	4.5
	Mean	4.4	4.5	4.5	4.5	
	C.D. (P=0.05)	Treatment = N.S.; Storage = N.S. ; Treatment × Storage = N.S.				
Ascorbic acid	Without honey	11.5	8.3	5.6	3.4	7.2
(mg/100ml)	With honey	11.3	8.3	5.3	3.4	7.2
	Mean	11.4	8.3	5.5	3.4	
	C.D. (P=0.05)	Treatment = N.S. ; Storage = 0.33 ; Treatment × Storage = 0.47				
Total Carotenoids	Without honey	2.23	2.08	1.44	1.00	1.69
(mg/100ml)	With honey	2.63	2.44	1.53	1.15	1.94
	Mean	2.43	2.26	1.48	1.08	
	C.D. (P=0.05)	Treatment = 0.10 ; Storage = 0.14 ; Treatment × Storage = 0.16				
Total Chlorophyll	Without honey	4.06	2.47	2.34	1.90	2.69
(mg/100ml)	With honey	5.11	3.33	2.48	2.01	3.23
	Mean	4.59	2.90	2.41	1.95	
	C.D. (P=0.05)	Treatment = 0.30 ; Storage = 0.43 ; Treatment × Storage = 0.49				
Total phenols	Without honey	18.2	16.8	14.0	12.3	15.4
(mg/100ml)	With honey	16.1	14.2	12.6	10.4	13.3
	Mean	17.2	15.5	13.3	11.4	
	C.D. (P=0.05)	Treatment = 0.49 ; Storage = 0.59 ; Treatment × Storage = 0.66				

Total chlorophyll (mg/100ml)

significantly higher total chlorophyll content was observed in squashes prepared with honey than without honey. The total chlorophyll content of squash prepared with honey (3.23 mg/100 ml) was more than those prepared without honey (2.69 mg/100 ml).

The greenish colour hue in some samples of honey depending on the source of extraction of honey might be the reason for apparently higher chlorophyll content in squash prepared with honey (Bogdanov *et al*., 2004). The colour change from bright green to olive brown during processing is attributed to conversion of chlorophylls to pheophytins due to the loss of central magnesium ion and due to internal changes caused by available headspace oxygen. Similar results were found by Gaur *et al.* (2007) in chlorophyll degradation kinetics of mint leaves puree. They reported that chlorophyll degradation followed the first order reaction kinetics. Chlorophyll in mint leaves puree was found to be most heat stable at pH 7.5 whereas at pH 6.5 and 8.5 the rate of chlorophyll degradation was found to be comparatively higher.

In the present study, the decrease in chlorophyll content during storage could also be due to degradation of chlorophyll. The acidic pH maintained in squash causes loss of chlorophyll 'a', 'b' and total chlorophyll because chlorophyll are stable in alkaline pH and acidic pH enhances its destruction. The degradation might also be affected by the temperature used for processing and storage conditions.

Changes in organoleptic evaluation (on 9 point hedonic scale) of green mango-mint-*Tulsi* squash with or without honey during storage:

The organoleptic evaluation analysis data is presented in Table 4. The data revealed that there was no significant change in colour and appearance (Fig. 1) of squashes during storage amongst the squashes prepared with or without honey.

It was observed that there was no significant change in flavour, taste, mouthfeel and overall acceptability scores of squashes during storage. However, throughout the storage period, higher flavour, taste, mouthfeel and overall acceptability scores were recorded for squashes prepared with honey as compared to without honey. The squashes remained highly acceptable for its all sensory parameters

Table 4: Changes in organoleptic evaluation of green mango-mint-*Tulsi* squash with or without honey during storage

Organoleptic evaluation	Storage / Treatment	0 day	30 days	60 days	90 days	Mean
Colour and	Without honey	7.83	8.00	7.67	7.83	7.83
appearance	With honey	8.00	7.83	7.33	7.67	7.71
	Mean	7.92	7.92	7.50	7.75	
	C.D. (P=0.05)	Treatment = N.S. ; Storage = N.S. ; Treatment × Storage = N.S.				
Flavour	Without honey	7.33	8.00	7.67	7.83	7.71
	With honey	8.33	8.67	8.33	8.33	8.42
	Mean	7.83	8.33	8.00	8.08	
	C.D. (P=0.05)	Treatment = 0.54 ; Storage = N.S. ; Treatment × Storage = N.S.				
Taste	Without honey	7.33	7.67	7.67	7.83	7.63
	With honey	8.33	8.67	8.33	8.33	8.42
	Mean	7.83	8.17	8.00	8.08	
	C.D. (P=0.05)	Treatment = 0.45 ; Storage = N.S. ; Treatment × Storage = 0.50				
Mouthfeel	Without honey	7.33	7.67	7.67	7.83	7.63
	With honey	7.83	8.33	8.33	8.33	8.42
	Mean	7.58	8.00	8.00	8.08	
	C.D. (P=0.05)	Treatment = 0.40 ; Storage = N.S. ; Treatment × Storage = 0.55				
Overall	Without honey	7.47	7.83	7.67	7.83	7.70
acceptability	With honey	8.13	8.37	8.07	8.17	8.18
	Mean	7.80	8.10	7.87	8.00	
	C.D. (P=0.05)	Treatment = 0.24 ; Storage = N.S. ; Treatment × Storage = N.S.				

NS= Non-significant

Fig 1: Colour of green mango-mint-*Tulsi* squash (a) with honey and (b) without honey

even after three months of storage.

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