Studies on preparation of acid lime squash J. N. PAPADE, P. M. CHANDAN, AND A. K. SAHOO

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ABSTRACT

The experiment was carried out during the year 2012-13 at Post Harvest Technology Laboratory, Department of Horticulture, Post Graduate Institute Dr. PanjabraoDeshmukh Krishi Vidyapeeth, Akola. Fully matured and uniform sized fruits of PDKV lime and seedless lime i.e. PDKV lime (V_v) and seedless lime (V_v) were procured from All India Co-ordinate Research Project on Citrus, Dr. PDKV, Akola and the squash was prepared by mixing of different amounts of lime juice and sugar as per treatments, Juice 25% + 40°B Sugar + 350 ppm KMS (T_v), Juice 25% + 42°B Sugar + 350 ppm KMS (T_v), Juice 25% + 40°B Sugar + 350 ppm KMS (T_v), Juice 25% + 40°B Sugar + 350 ppm Sodium benzoate (T_v), Juice 25% + 40°B Sugar + 350 ppm Sodium benzoate (T_v), Control- Juice 25% + 45°B Sugar (T_v). Then the squash was treated with preservative KMS or Sodium benzoate (T_v), Control- Juice 25% + 45°B Sugar (T_v). Then the squash was treated with preservative KMS or Sodium benzoate at 350ppm concentration. The observations in respect to chemical analysis and sensory evaluation of lime squash was recorded periodically at an interval of 20 days upto 80 days and the data obtained was analyzed using FCRD design. A gradual increase in TSS, reducing sugar and total sugars content of the acid lime squash was observed on advancement of storage period irrespective to varieties and treatments used However, titratable acidity content, non-reducing sugars and ascorbic acid content of squash were found to decrease with the advancement of storage period. The acid lime squash prepared from variety seedless lime was found to be acceptable up to 60th days of storage in respect to sensory qualities whereas acid lime squash prepared from variety PDKV lime was found to be acceptable 80th days of storage period. So the treatment combination V_1T_3 was concluded as the best treatment all variety and treatment combination.

Keywords: Chemical analysis, PDKV lime, seedless lime, sensory evaluation, squash

Acid lime (Citrus aurantifolia Swingle) belongs to family rutaceae is the third important citrus fruit crop in India next to mandarins and sweet oranges. This citrus species is grown in every state of India, but the leading producer states are Andhra Pradesh, Maharashtra, Assam and Karnataka (Rao, 2008). Out of the total production, only 1 per cent acid lime fruits is used for processing and approximately 0.5 per cent processed products of acid lime are exported to the other countries. Acid lime is not eaten directly due to sour in taste, but can be consumed as squash. Fruits, whether fresh or dried, have always formed a part of the staple diet of human beings. The reason for this is that, they are rich in nutrients and provides some of the essential minerals and vitamins which are useful to our body. Fruit is rich in vitamin C, excellent source of calcium, phosphorus, iron. Nutritive value derived from 100 ml juice of acid lime represents protein 1.5%, fat 1.0%, fiber 1.3%, minerals 0.7%, carbohydrates and 10.9%, vitamin C 63 mg, iron 0.3%, calcium 90 mg, phosphorus 20 mg and moisture 84.6%. Besides, high nutritive value, it is well for its excellent medicinal properties. Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties, but it is a natural antiseptic and smells divine. It is a digestive stimulant and improves both digestion and appetite. Lime juice added to one meal each day helped protect people from contracting cholera, cramping. The vitamin C in lime juice boosts immune function and acts as one of the most powerful dietary antioxidants. Lime has been shown to have both Email: peterson2702@gmail.com

medicinal and cosmetic values. Lime juice benefits are very diverse, Lime juice can be used as a flavoring food, beverages, refreshments, citric acid preservative and cleaning up the rust on the dirty metal and leather.

Amongst the various fruits particularly grown in the tropical and subtropical regions, acid lime is one of the important commercial fruit crop which having the excellent processing qualities. Being non climacteric fruits, acid lime cannot be stored for a long period. Limes can be kept out at room temperature where they will stay fresh for up to one week. Therefore, the development of appropriate processing technology and product standardization will definitely help in better utilization of acid lime fruits particularly during the seasonal glut. The processed products prepared from well matured acid lime fruits are pleasant, best flavored, good storable and eventually represents better value added form that aids to conserve the excess fruit production and fast perishability of the fruits.

MATERIALS AND METHODS

The research was done at Department of Horticulture, Dr. Panjabrao Krishi Vidya Peeth, Akola, Maharashtra during 2012-2013. Fully matured and uniform sized fruits of acid lime *i.e.* PDKV lime (V_1) and Seedless lime (V_2) were procured from All India Coordinate Research Project on Citrus, Dr. PDKV, Akola. Juice was extracted manually from well matured fruits. Juice obtained from two different varieties ware treated with various concentrations of sugar and preservatives separately as following, Juice 25% + 40°B Sugar + 350

J. Crop and Weed, 11(1)

ppm KMS (T₁), Juice $25\% + 42^{\circ}B$ Sugar + 350 ppm KMS (T₂), Juice $25\% + 45^{\circ}B$ Sugar + 350 ppm KMS (T₃), Juice $25\% + 40^{\circ}B$ Sugar + 350 ppm Sodium benzoate(T_4), Juice 25% + 42°B Sugar + 350 ppm Sodium benzoate (T₅), Juice $25\% + 45^{\circ}B$ Sugar + 350 ppm Sodium benzoate (T₆), Control- Juice $25\% + 45^{\circ}B$ Sugar (T_2) . For formulation of above mention recipes, the actual total soluble solids and acidity present in the Juice was first determined and then remaining quantity of sugar were adjusted in 1000 ml. Squash of each recipe was prepared by mixing the calculated quantity of juice, sugar and water. At first sugar syrup was prepared by heating the mixture of sugar and water then squash was prepared with fruit juice as per the given recipes. The prepared squash was then filtered properly through a clean muslin cloth to obtain a final product of uniform consistency. The products were poured into sterilized bottles, of 150 ml capacity. After leaving 2 cm head space, bottles were crown corked by automatic crown corking machine. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with three replications. After preparation of squash, the chemical parameters like total soluble solids, titratable acidity, ascorbic acid, total sugar, reducing sugar, nonreducing sugar were analyzed by the methods described by Rangana (1979). The sensory panel consisted of 5 trained panelists and they evaluated the experimental samples as per the hedonic scale. The mean values of score for sensory evaluation were calculated and reported according to the method reported by Amerine et al. (1965).

RESULTS AND DISCUSSION

Both the variety has significant impact on quality of lime squash. There was increasing trend of total soluble solids content in acid lime squash during the storage period. Significantly minimum TSS 38.03% was observed at 1st day, which was increased to 38.76% on 80th day of storage in PDKV lime squash. Whereas in seedless lime squash, TSS was increased from 37.58% (at 1st day) to 39.25% (at 80th day) during storage. Thus minimum increase (0.73%) in total soluble solid during 80 days of storage was noticed in PDKV lime squash. However, maximum change (1.67%) was noticed in seedless lime squash.

The minimum increased in TSS from 37.81% (at 1^{st} day) to 38.36% (at 80th day) was observed in treatment T₁. Whereas maximum increased in TSS was noticed in T₃ from 35.51% (at 1^{st} day) to 37.90% (at 80th day) during storage. Thus minimum increase (0.55%) in total soluble solid during 80 days of storage was noticed in T₁. Whereas, maximum change (2.39%) was noticed in T₃.

Increase in TSS content of squash might be due to conversion of polysaccharide into sugars during the process of hydrolysis. The observations analogues to these findings was also reported by Kumar *et al.* (2001) in aonla squash, Kannan and Thirumaran (2001) in jamun squash and Nath *et al.* (2005) in ginger kinnow squash.

The acid lime squash prepared by using variety PDKV lime squash showed less change in total soluble solids at 80 days of storage as compared to seedless lime squash. Minimum (35.43%) total soluble solids of acid lime squash observed in treatment combination V_1T_6 at 1st day which was increased to 35.97% 80 days of storage. Whereas, Maximum changes in total soluble solids of acid lime squash was observed in treatment combination V_1T_3 35.93% at 1st day which was increased to 38.49% at 80 day of storage.

The titratable acidity of acid lime squash was decreased in both the variety during storage period. At the 1st day, acidity in PDKV lime squash was 0.87% which was reduced to 0.83% at 80th day of storage. Whereas in Seedless lime, at the 1st day acidity was 0.91% which was reduced to 0.86% at 80th day of storage. So, 0.04% decreased in acidity was observed in V₁ whereas 0.05% acidity was deceased in V₂.

PDKV and seedless lime exhibited significant decreased in titratable acid content of squash during storage period. At the 1st day minimum acidity 0.89% was recorded in T_2 which was decreased to 0.79 at 80th day of storage. Whereas maximum decreased in titratable acidity was observed in T_3 . At the 1st day, acidity was 0.87% which was decreased to 0.79% at 80th day of storage.

Acidity in lime squash was decreased during storage period. This might be attributed to hydrolysis of polysaccharides, where organic acid is converting to hexose sugar or complexing in the presence of metal ions. During storage, the decreased in acidity during storage was observed in mango squash (Roy *et al.*, 1997), in kinnow squash (Sogi *et al.*, 2001) and in aonla squash (Jain *et al.*, 2006).

Minimum rate of decreased in acidity was observed in treatment combination V_1T_5 . At the 1st day acidity was 0.92% which was reduced to 0.89% at 80th day of storage. Whereas, maximum rate of decreased was recorded in treatment combination V_1T_3 .

Declining trend might be due to chemical interaction between the chemical constituent of juice include by temperature influencing enzymatic action (Palaniswamy and Muthukrishnam, 1974 and Sethi, 1993) in litchi squash and kumar *et al.* (2001) in aonla squash also observed similar result that showed decrease in acidity content during storage.

Preparation of acid lime squash

The reducing sugar of acid lime squash was increased during storage period upto 80 days in both varieties. The maximum percentage (12.31%) increased in reducing sugar was observed in the variety PDKV lime squash whereas Seedless lime recorded a lower percentage (10.48%) increased in reducing sugar.

At the 1st day of storage maximum (17.86%) reducing sugar was recorded in treatment T₃ which was increased to 33.92% at 80th day of storage. The treatment T₃ showed the maximum percentage (16.06%) increased in reducing sugar. Whereas, minimum percentage (6.76%) increased in reducing sugar was found in treatment T₇.

The data representing the interaction effects of variety and different treatments on reducing sugars content of acid lime squash was significantly increased from 0 to 80 days of storage. Maximum reducing sugar was noticed in the treatment combination V_1T_2 (15.32 %) followed by treatment combination V_2T_3 (17.37 %) at the 1st day of storage. The reducing sugar was continuously increased during storage period and maximum (31.39%) was found in treatment combination V_1T_2 . Whereas, minimum % of reducing sugars was observed in treatment combination V_2T_6 (13.20 %) at 1st day of storage.

Table 1: Varietal effect on quality parameters of acid lime squash

Varieties	TSS	Acidity	Reducing	Non reducing	Total sugar	Ascorbic acid
	([°] brix)	(%)	sugar (%)	sugar (%)	(%)	(mg 100 g ⁻¹)
V_1	38.76	0.83	27.20	18.80	46.01	30.42
V_2	39.25	0.86	24.26	17.89	42.16	30.35
SEm (±)	0.13	0.001	sig	sig	sig	0.001
LSD (0.05)	0.40	0.005	0.43	0.87	0.95	0.003

Varieties	TSS ([°] Brix)	Acidity (%)	Reducing sugar (%)	Non reducing sugar (%)	Total sugar (%)	Ascorbic acid (mg 100 g ⁻¹)
T ₁	38.36	0.82	26.68	18.25	44.93	30.93
T ₂	39.35	0.85	28.28	19.02	47.30	30.95
T ₃	37.90	0.79	33.92	20.67	54.59	30.95
T ₄	38.46	0.85	25.62	17.97	43.59	30.91
T ₅	39.13	0.88	22.21	17.61	39.82	29.41
T ₆	40.93	0.83	22.73	17.98	40.71	30.05
T ₇	38.89	0.85	20.73	16.96	37.69	29.51
SEm (±) LSD (0.05)	0.26 0.78	0.003 0.009	0.73 2.16	1.50 4.40	6.64 4.80	0.002 0.006

Table 2: Treatment observation in quality parameter

The increase in reducing sugars might be due to the conversion of non-reducing sugar to reducing sugar owing to the process of hydrolysis. Palaniswamy and Muthukrishanan (1974) noted the increased in reducing sugars in lemon juice with the advancement of storage period. Mehta and Bajaj (1983) have suggested that the increase in reducing sugars of aonla syrup during storage was probably due to gradual inversion of non-reducing sugars. Jain *et al.* (1984) reported that increased in reducing sugar in lemon, orange and bael squash during storage period.

The non-reducing sugar of acid lime squash was found to decrease during storage period up to 80 days at both the variety. The maximum (0.89%) increase in non-reducing sugar was recorded in PDKV lime (V_1). At the 1st day of storage non-reducing sugar was 19.69% and this was decreased to 18.80% at 80th day of storage.

Whereas, minimum 0.73% decreased in non-reducing sugar was found in Seed less lime (V₂).

At the 1st day of storage maximum (21.52%) nonreducing sugar was recorded in treatment T₃ which was decreased to 20.67% at 80th day of storage. Whereas, minimum (17.87%) was found in treatment T₇ at the 1st day of storage and it was decreased to 16.96% at the 80th day of storage. However, maximum (0.93%) decreased in non-reducing sugar was recorded in treatment T₂.

Increase in reducing sugars and decrease in nonreducing sugars during storage is a general phenomenon as noticed by Roy *et al.* (1979) in bael squash. Reddy and Chikkasubbanna (2008) also recorded decline in non-reducing sugar during storage of lime blended aonla squash. On the advancement of storage period, non-reducing sugar content of lime squash was also decreased gradually. At the 1st day of storage maximum (22.65%) non-reducing sugar was recorded in treatment combination V_1T_3 which was decreased to 21.64% on 80th day of storage. Whereas, minimum (17.54%) non-reducing sugar was observed in treatment combination V_2T_7 at the 1st day of storage and it reduced to 16.60% at 80th day of storage. However maximum (1.16%) of decreased in non-reducing sugar was recorded in treatment combination V_1T_2 and minimum (0.47%) decreased in non-reducing sugar was observed in treatment combination V_1T_5 .

The process of hydrolysis of polysaccharides sugars into reducing sugars was faster at ambient storage conditions. This might be due to the slow rate of conversion of sugar as it influenced by low temperature. The results mentioned above are in conformity with the findings of various research workers. Decrease in nonreducing sugars is a general phenomenon as noticed by Roy *et al.* (1979) in bael squash.

There was increased in total sugars of acid lime squash during 80 days of storage period. Highest (34.59%) total sugar was found in variety PDKV lime (V_1) whereas lowest (32.41%) total sugar was recorded in V_2 . However maximum percentage increased in total sugar was observed in V_1 which was 11.42%.

At the 1st day of storage maximum (39.38%) total sugar was recorded in treatment T_3 and minimum (31.83%) was found in treatment T_7 . On the 80th day of storage, similarly T_3 had maximum (54.59%) total sugar and T_7 had minimum (37.69%). However, maximum (15.21%) changed in total sugar was observed in treatment T_3 and minimum (5.86%) increased in total sugar was found in T_7 .

At the 1st day of storage maximum (40.99%) total sugar was found in treatment combination V_1T_3 and minimum (30.74%) total sugar was found in treatment combination V_2T_4 . However, the total sugar was increased gradually on advancement of storage period. At the 80th day of storage, maximum (55.85%) sugar was found in treatment combination V_1T_3 but minimum (35.88%) total sugar was found in treatment combination V_2T_7 . Whereas, maximum (15.56%) increased in total sugar was found in treatment combination V_2T_3 and minimum (4.67%) increased in total sugar was found in treatment combination V_2T_7 .

The increased in total sugars of acid lime squash during storage was probably due to conversion of starch and pectin into simple sugars. Mehta and Bajaj (1983) reported that, there was gradual increase in total sugars content of citrus juices. Jain *et al.* (2006) reported that, there was slight increase in total sugars of aonla squash ranged from 45.54 to 46.75 per cent. They further reported that these changes in sugar content might be due to partial hydrolysis of complex carbohydrate and also hydrolysis must have been accelerated due to high temperature and lowest humidity in the atmosphere.

The experimental data showing a significant decrease in ascorbic acid content in acid lime squash during storage period irrespective to varieties and treatments. At the 1st day of storage highest ascorbic acid (30.54mg 100g⁻¹) was found in variety PDKV lime (V₁) and lowest (30.48 mg 100g⁻¹) was found in Seedless lime. Whereas, at 80th day of storage, similarly maximum ascorbic acid (30.42 mg 100g⁻¹) was found in variety PDKV lime (V₁) and lowest (30.35 mg 100g⁻¹) was found in variety PDKV lime (V₁) and lowest (30.35 mg 100g⁻¹) was found in variety PDKV lime (V₁) and lowest (30.35 mg 100g⁻¹) was found in Seedless lime. However, maximum (0.13 mg 100g⁻¹) decreased in ascorbic acid was found in V₂ and minimum (0.12 mg 100g⁻¹) decreased was observed in V₁.

Maximum ascorbic acid (31.14%) content was observed in treatment T₃ and minimum ascorbic acid (29.51 mg 100g⁻¹) was found in treatment T₅ at the 1st day of storage. Whereas on 80th day of storage maximum ascorbic acid (30.95 mg 100g⁻¹) was found in treatment T₃ and minimum ascorbic acid (29.41mg 100g⁻¹) was found in treatment T₅. However, maximum decreased (0.19%) in ascorbic acid was found in treatment T₃ and minimum decreased (0.10%) in ascorbic acid was found in treatment T₅.

At the 1st day of storage maximum (31.15mg 100g⁻¹) ascorbic acid was found in treatment combination V_1T_3 and minimum (29.50mg 100g⁻¹) ascorbic acid was found in treatment combination V_2T_5 . However, the ascorbic acid was decreased gradually on advancement of storage period. At the 80th day of storage, maximum (30.96 mg 100g⁻¹) ascorbic acid was found in treatment combination V_1T_3 but minimum (29.35mg 100g⁻¹) ascorbic acid was found in treatment (0.59%) decreased in ascorbic acid was found in treatment (0.09%) decreased in ascorbic acid was found in treatment combination V_2T_5 .

Bhatia (1995) similarly observed a continuous decreased in ascorbic acid content of jackfruit squash packed under various treatments, with rate of decreased being maximum at 37°C and minimum at 2-5°C. Sahoo *et al.* (2014) suggested the decreased in ascorbic acid content on advancement of storage period in banana powder.

Preparation of acid lime squash

As quality is the ultimate criterion of desirability of any product, the overall quality of lime squash depend on the nutritional and sensory quality as assessed by the sensory panel. The overall acceptability of the lime squash depends upon colour, taste and flavour from defects as per remarks of the evaluator. The sensory quality was gradually decreased on the advancement of storage period.

At the 1st day of storage maximum score '8' on quality was found in treatment combination V_1T_3 whereas lowest score '5.7' was observed in treatment combination V_2T_6 . Again at 60th day of storage maximum score '7.6' on quality was found in treatment combination V_1T_3 whereas lowest score '5.0' was observed in treatment combination V_2T_6 . After 60 days the quality of V_2T_2 and V_2T_6 was not acceptable. The products were damaged. But out of remaining samples, V_1T_3 secured highest score '7.3' whereas lowest acceptance '5.8' was found in V_1T_6 , V_2T_5 and V_2T_7 . So out of all treatment combinations, V_1T_3 was found to be the best in terms of quality.

The sensory score decreased continuously with increase in storage period .This might be due to degradation of volatile substances and flavor constituents. The decreasing trend was observed for taste, flavor and color with increase in storage .Loss in

Treatment combinations	TSS ([°] brix)	Acidity (%)	Reducing sugar (%)	Non reducing sugar (%)	Total sugar (%)	Ascorbic acid (mg 100 g ⁻¹)	
V ₁ T ₁	38.91	0.80	29.23	18.74	47.98	30.75	
V_1T_2	39.85	0.83	31.79	19.48	51.27	30.61	
V_1T_3	38.49	0.78	34.21	21.64	55.85	30.96	
V_1T_4	38.98	0.82	24.79	18.25	43.04	30.89	
V_1T_5	39.82	0.89	23.11	17.75	40.87	29.35	
V_1T_6	35.97	0.80	25.15	18.43	43.59	30.23	
V_1T_7	37.87	0.85	22.17	17.33	39.50	29.53	
V_2T_1	37.81	0.84	24.13	17.75	41.87	30.80	
V_2T_2	38.86	0.85	24.76	18.57	43.33	30.50	
V_2T_3	40.97	0.81	33.63	19.70	53.33	30.94	
V_2T_4	37.95	0.87	26.46	17.69	44.14	30.70	
V_2T_5	40.90	0.89	21.30	17.47	38.77	29.39	
V_2T_6	39.85	0.85	20.31	17.52	37.84	29.86	
V_2T_7	40.91	0.88	20.28	16.60	35.88	29.50	
SEm (±) LSD (0.05)	0.35 1.06	0.005 0.013	1.04 3.04	2.13 6.22	2.33 6.80	0.003 0.009	

Table 3: Interaction effects of varieties and treatments in quality parameter

Table 4: Effect of varieties and treatments on sensory characteristics of acid lime squash during storage

Treat		20 D	ay		60 Day				80 Day			
-ment	Color	Flavor	Taste	Average	Color	Flavor	taste	Average	Color	Flavor	taste	Average
V_1T_1	7.0	7.0	7.5	7.2	7.0	6.0	6.0	6.3	6.5	6.0	6.0	6.2
V_1T_2	7.0	7.0	7.5	7.1	6.0	7.0	7.0	6.7	6.0	6.0	6.0	6.0
V_1T_3	8.0	8.0	7.5	8.0	8.0	8.0	7.0	7.6	7.0	8.0	7.0	7.3
V_1T_4	6.0	7.0	7.0	6.7	6.0	6.5	7.5	6.5	6.0	6.5	6.0	6.2
V_1T_5	7.0	6.5	6.0	6.5	6.0	6.5	6.5	6.3	6.0	6.5	6.0	6.2
V_1T_6	7.0	6.5	7.0	6.8	6.0	6.5	6.0	6.2	6.0	6.5	5.0	5.8
V_1T_7	8.0	7.0	7.0	7.3	6.0	7.0	8.0	7.0	6.0	7.0	7.0	6.7
$V_{2}T_{1}$	7.0	7.0	8.0	7.3	6.0	7.0	7.5	6.8	6.0	5.5	7.0	6.2
V_2T_2	7.0	6.0	8.0	7.0	7.0	6.0	7.0	6.7	*	*	*	*
V_2T_3	7.5	7.0	7.5	7.3	7.0	7.0	6.5	6.8	5.5	6.5	6.0	60
V_2T_4	7.0	7.0	7.5	7.2	7.0	6.0	7.0	6.7	6.0	6.5	5.5	6.0
V_2T_5	6.0	7.0	7.0	6.7	6.0	6.0	7.0	7.3	5.0	6.0	6.5	5.8
V_2T_6	5.0	6.0	6.0	5.7	4.0	5.0	6.0	5.0	*	*	*	*
$V_{2}T_{7}$	7.0	7.0	8.0	7.3	7.0	6.0	7.0	6.7	5.5	6.0	6.0	5.8

Note :* The quality parameters were not acceptable

sensory quality and storability of product after certain period is obviously happened in temperature also play an important role in inducing certain biochemical changes in the products which leads to discoloration thus masking of the original color of the products appeared.

Similar result was also reported by Palniswamy *et al.*(1974) in mango squash. Mehta and Bajaj (1983) in Kinnow Mandarin and blood orange squash. Kumar *et al.* (2001) in ginger Kinnow squash and Srinivas *et al.*(2007) in pomegranate squash.

During storage period there was continuous decrease in ascorbic acid, titratable acidity and nonreducing sugar whereas reducing sugar, TSS and total sugar was increased. However among varieties, PDKV lime (V₁) was found superior as it contained more reducing sugar, non-reducing sugar, total sugar and ascorbic acid which indicating the better content of nutrients in PDKV lime. Among different treatments T₃ (Juice $25\% + 45^{\circ}B$ Sugar + 350 ppm KMS) was observed as best treatment because it was rich in nutrients like reducing sugar, non-reducing sugar, total sugar and ascorbic acid. In sensory evaluation treatment combination V₁T₃ secured highest score in quality evaluation. Again the acid lime squash prepared from variety seedless lime was found to be acceptable up to 60th days of storage in respect to sensory qualities whereas squash prepared from variety PDKV lime was found to be acceptable upto 80th days of storage period. So the treatment combination V_1T_3 was concluded as the best treatment all variety and treatment combination.

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