



Studies on biochemical constituents of sapota (*Manilkarazapota* L.) at different stages of ripening during storage

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Abstract: Sapota fruits (Var. Cricket Ball) of three different ripening stages i.e. mature (≥ 13 kg/cm²), half ripe (6-7 kg/cm²), and full ripe (2-3 kg/cm²), were packed with newspaper soaked with 3 dosages (1000 ppm, 2000 ppm and 3000 ppm) of ethylene absorbent (KMnO₄) along with control and stored at normal room temperature by packing in cardboard boxes. It was observed that specific gravity, ascorbic acid and total phenols decreased whereas cumulative loss in weight and malondialdehyde content increased with increasing period of storage. Fruits packed in different concentrations of KMnO₄ soaked paper had less cumulative loss and retained more content of specific gravity, ascorbic acid and total phenols in all three stages. But less malondialdehyde content was observed. The effect of KMnO₄ increased with increasing concentrations of KMnO₄ in all three stages. As a result, more desirable content was noted in fruits packed with 3000 ppm concentration of KMnO₄ in mature fruits. This work relates to enhancement of shelf life of sapote so that it may be transported to far off places.

Keywords: KMnO₄, Ripening stages, Sapota, Specific gravity, Total phenols

INTRODUCTION

Sapota (*Manilkarazapota* L.) also known as the sapodilla, is one of the important fruits grown in India. The area and production of Sapota in India during 2014-15 was estimated to be about 1,06,490 hectare and 13,39,010 MT and in Haryana, it was estimated about 1,600 hectare and 13,920 MT (Gandhi, 2015), respectively. It is reported to contain digestible sugar (12-20%) (SiddapaandBhatia, 1954), acids (Shanmugavelu and Srinivasan, 1973), proteins, amino acids, carotenoids, ascorbic acid and minerals like potassium, calcium and iron (SelverajandPal, 1984). Fruits are climacteric in nature and highly perishable as results of which its shelf-life is very low (Qurohsian and Meah, 1991). After harvest, fruits undergo many physiological and biochemical changes because of its living entity and high water content. Apart from those changes, microbial cumulative also contributes to postharvest losses during ripening and storage. The storage life of a fruit could be prolonged significantly through slowing down the ripening process and controlling the microbial cumulative.

The ripening is mainly because of production of ethylene. Excess of ethylene leads to the ripening at earlier stage and thus the fruit cumulates immediately. Use of ethylene absorbent absorb the released ethylene and thus slow down the process of ripening. The delay in the ripening process helps to enhance the shelf-life of fruit. However, appropriate concentration of

KMnO₄ (ethylene absorbent) is must for increasing the shelf life of fruits. Storage quality of sapota is highly dependent upon the stage of harvesting. Generally, sapota when harvested at later stages had reduced shelf life, while early harvesting caused more weight loss but showed better storability. However, no systematic studies have been reported in literature about effect of ethylene absorbent on different ripening stages of Sapota. Therefore, the present investigation was planned to evaluate the effect of different concentration of KMnO₄ on different ripening stages of sapota.

MATERIALS AND METHODS

Experimental Site: Present experiment was conducted in the laboratory of department of Botany & Plant Physiology, CCS, Haryana Agricultural University, Hisar.

Collection of samples: Fully mature fruits of sapota were harvested from the orchard of department of Horticulture, CCS, Haryana Agricultural University, Hisar with the secateurs keeping small intact pedicel with each fruits.

Experimental set-up: Fruits were harvested and divided into 3 lots. One lot of mature fruit was stored as such after harvest by giving different treatments of KMnO₄. Second lot was stored at room temperature till fruits were half ripe and then different treatments were given and were stored at room temperature. Whereas 3rd lot of fruits was stored at room temperature till full

ripe stage and then different treatments were given as in lot 1 and lot 2. KMnO₄ was applied at the rate of 0 (control), 1000 ppm, 2000 ppm and 3000 ppm in soaked newspapers (Sharma *et al.*, 1992). Desired concentrations of KMnO₄ were prepared and newspaper was dipped in that solution. These dipped newspapers were dried in shade. Fruits after packing in desired concentrations of KMnO₄ soaked paper were packed in cardboard boxes. Two kg. of fruits were packed in cardboard boxes and treated as one replicate. All the treatments were replicated four times for taking the various observations. Fruits of three replications were weighted on alternate days for recording the cumulative loss whereas three fruits were taken at random from fourth replication and were analysed for various quality parameters such as ascorbic acid, specific gravity, total phenols and malondialdehyde etc. on alternate days of storage (i.e. 0, 2nd, 4th, 6th, 8th and 10th in mature fruits, 0, 2nd, 4th, and 6th day in half ripe fruits and 0, 2nd and 4th day in full ripe fruit). The experiment was designed in completely randomized design.

Analytical methods: Cumulative loss is the total loss including physiological loss in weight PLW and decay loss. Calculation of PLW: The fruit weight was determined by weighing the fruits of three replicates on each day of observation. The physiological loss in weight was taken on alternate day of observation and calculated by the following formula suggested by Srivastava and Tandon (1968).

Physiological loss in weight (%) = $\frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$

Calculation of decay loss: Fruits showing rotting due to micro-organisms infection were considered as decayed ones and were weighed on alternate day of observation and were removed from the box. The percent decay loss was estimated by the following formula suggested by Srivastava and Tandon (1968).

Decay loss (%) = $\frac{(W-w)}{W} \times 100$

Where, W= Total weight of the fruits, w = Weight after eliminating decayed fruits

Calculation of ascorbic acid: Ascorbic acid was determined by the titration method of AOAC (1990).

Ascorbic acid (mg/100g) = $\frac{\text{Titer value} \times \text{Total volume made up}}{\text{Standard reading} \times \text{Volume taken} \times \text{Weight of sample taken}} \times 100$

The results were calculated and expressed as mg of ascorbic acid per 100 g of fruit pulp.

Calculation of specific gravity: Specific gravity was calculated by dividing the weight of the fruit with the volume of the same fruit.

Calculation of total phenols: The total phenols in the fruit tissue were estimated by method given by Amorium *et al.* (1977) using Folin-ciocalteau's reagent and modified by Tung *et al.* (2007). Values obtained were expressed in mg/g total phenols.

Calculations of Malondialdehyde: Malondialdehyde (MDA) is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968). The concentration of MDA was calculated using its extinction coefficient of 155 mm⁻¹cm⁻¹. Observations were tabulated and statistically analysed.

RESULTS AND DISCUSSION

Cumulative loss in weight: Data presented in table 1 predicts that cumulative loss in weight of fruits increased with the increase in storage period irrespective of treatments at all stages. Cumulative loss increased from 27.37% to 37.85% after 10th day of storage in the mature fruits, from 9.51% to 34.21% after 6th day of storage in half ripe fruits and 19.34% to 34.12% after 4th day of storage in full ripe fruits similar results in recent issue of this journal Kumari *et al.*, 2017. Higher water content in the fruits might have been responsible for higher rate of physiological processes and the attack of micro-flora (Because micro-flora generally

Table 1. Effect of various concentrations of ethylene absorbent (KMnO₄) on cumulative loss in weight (%) in Sapota fruits of different ripening stages during storage at room temperature.

KMnO ₄	Days of storage									
	Mature					Half ripe			Full ripe	
	2	4	6	8	10	2	4	6	2	4
Control	7.89	14.74	23.49	30.88	37.85	9.51	21.00	34.21	19.34	34.12
1000 ppm	6.95	12.87	20.35	27.62	35.18	8.18	16.78	29.73	15.98	31.09
2000 ppm	5.97	11.89	19.67	24.35	33.34	7.17	12.63	22.93	13.70	28.81
3000 ppm	4.76	9.36	15.26	19.93	27.37	5.02	10.73	20.08	10.85	25.78
CD at 5%	0.40	0.85	1.80	1.91	2.21	0.13	1.02	1.62	1.95	2.90

Table 3. Effect of various concentrations of ethylene absorbent (KMnO₄) on specific gravity in Sapota fruits of different ripening stages during storage at room temperature.

KMnO ₄	Days of storage															
	Mature							Half ripe					Full ripe			
	0	2	4	6	8	10	Mean	0	2	4	6	Mean	0	2	4	Mean
Control	1.13	1.09	1.07	1.05	1.01	0.93	1.04	1.08	1.05	1.01	0.93	1.02	1.05	0.98	0.94	0.99
1000ppm	1.13	1.09	1.07	1.07	1.02	0.95	1.05	1.08	1.07	1.03	0.93	1.03	1.05	1.00	0.97	1.01
2000ppm	1.13	1.10	1.09	1.08	1.04	0.98	1.07	1.08	1.07	1.04	0.95	1.04	1.05	1.03	0.98	1.02
3000ppm	1.13	1.12	1.10	1.08	1.05	1.01	1.08	1.08	1.07	1.05	0.99	1.05	1.05	1.03	0.99	1.02
Mean	1.13	1.11	1.08	1.07	1.03	0.96		1.08	1.06	1.03	0.96		1.05	1.01	0.97	

Table 4. Effect of various concentrations of ethylene absorbent (KMnO₄) on total phenols (mg/g) in Sapota fruits of different ripening stages during storage at room temperature.

KMnO ₄ (T)	Days of storage (D)																	
	Mature							Half ripe					Full ripe					
	0	2	4	6	8	10	Mean	0	2	4	6	Mean	0	2	4	Mean		
Control	1.54	1.36	1.28	1.19	1.11	1.06	1.25	1.28	1.21	1.12	1.04	1.63	1.17	1.09	1.02	1.09		
1000ppm	1.54	1.41	1.34	1.23	1.18	1.10	1.30	1.28	1.23	1.14	1.05	1.18	1.17	1.10	1.04	1.10		
2000ppm	1.54	1.44	1.41	1.34	1.25	1.19	1.16	1.28	1.24	1.16	1.07	1.19	1.17	1.12	1.06	1.12		
3000ppm	1.54	1.51	1.49	1.42	1.32	1.26	1.42	1.28	1.26	1.19	1.10	1.21	1.17	1.14	1.09	1.13		
Mean	1.54	1.43	1.38	1.30	1.22	1.15		1.28	1.24	1.15	1.07		1.17	1.12	1.05			
CD at 5%	T=0.03			D=0.04			T×D=N.S.			T=0.03			D=0.03			T×D=N. S.		

Table 5. Effect of various concentrations of ethylene absorbent (KMnO₄) on lipid peroxidation (Malondialdehyde) (nmol/g fw) content in Sapota fruits of different ripening stages during storage at room temperature.

KMnO ₄ (T)	Days of storage (D)																	
	Mature							Half ripe					Full ripe					
	0	2	4	6	8	10	Mean	0	2	4	6	Mean	0	2	4	Mean		
Control	2.28	2.58	2.97	3.36	3.60	4.35	3.19	2.72	3.22	3.67	4.46	3.51	3.38	3.72	4.51	3.87		
1000 ppm	2.28	2.54	2.81	3.04	3.27	3.96	2.97	2.72	3.18	3.58	4.21	3.42	3.38	3.66	4.38	3.81		
2000 ppm	2.28	2.49	2.75	2.98	3.14	3.78	2.90	2.72	3.13	3.47	4.08	3.35	3.38	3.62	4.13	3.71		
3000 ppm	2.28	2.47	2.61	2.87	3.04	3.60	2.81	2.72	3.06	3.41	3.98	3.29	3.38	3.55	3.97	3.63		
Mean	2.28	2.52	2.79	3.06	3.26	3.92		2.72	3.15	3.53	4.18		3.38	3.63	4.25			
CD at 5%	T=0.04			D=0.05			T×D=0.09			T=0.10			D=0.10			T×D=0.19		

flourish in high humidity Dennis, 1977. Increase in cumulative loss in weight during storage at room temperature (from 8.04 to 31.3% within 8 days) has also been reported by Nath *et al.* (2012) in Pear and Xing *et al.* (2015) in Jujube fruits. On 2nd day of storage, fruits of mature stage had minimum cumulative loss in weight when packed in 3000 ppm KMnO₄ soaked paper i.e. 4.76% whereas maximum cumulative loss in weight was observed in control fruits i.e. 7.89%. All the treatments of KMnO₄ were effective to decrease the cumulative loss in weight and the effect of KMnO₄ increased with increasing concentrations of KMnO₄. Same effect was observed on 4th, 6th and 8th day of storage. Maximum cumulative loss in weight was observed in control fruits (37.85%) after 10 days of storage in mature fruits whereas minimum cumulative loss in weight was observed in fruits packed in 3000 ppm KMnO₄ (27.37%) on same day of storage. In case of half ripe fruits, cumulative loss in weight increased from 2nd day (5.09%) to 6th day (20.08%) in case of control fruits whereas minimum increase in cumulative loss in weight was observed in fruits packed with 3000 ppm KMnO₄ soaked paper i.e. from 2nd day (5.02%) to 6th day (20.08%). In full ripe stage sapota fruits, cumulative loss in weight increased from 19.34% to 34.12% on 2nd and 4th day respectively, in control fruits and from 10.85% (2nd day) to 25.78% (4th day) in fruits packed with 3000 ppm KMnO₄ soaked paper. Cumulative loss in weight decreased with increasing concentrations of KMnO₄ on each day of storage. Decrease in concentration of ethylene by KMnO₄ (ethylene absorbent) might have reduced the process of softening (ripening stage) in fruits and thus decreased the attack of micro flora on the fruit surface. These results are in conformity with the results reported by Khosravi *et al.* (2015) in Apple, and Nath *et al.* (2015) in Tomato. Khosravi *et al.*, 2015 reported the effect of KMnO₄ (5g) in apple and similar

finding were absorbed.

Ascorbic acid: Ascorbic acid of fruits decreased with the increase in storage period irrespective of treatments at all stages as presented in Table 2. Ascorbic acid decreased from 23.68 mg/100g to 4.79 mg/100g after 10th day of storage in the mature fruits, from 12.87 mg/100g to 3.90 mg/100g after 6th day of storage in half ripe fruits and 9.08 mg/100g to 3.75 mg/100g after 4th day of storage in full ripe fruits. The decreasing trend in ascorbic acid content during storage could be due to the oxidation and conversion of L-ascorbic acid into dehydro-ascorbic acid in presence of enzyme ascorbic acid oxidase (Nayak *et al.*, 2011). Nath *et al.* (2012) in tomato and Shahi *et al.* (2015) in ber also observed decrease in ascorbic acid content (30mg/100g to 5 mg/100g) during storage. On 2nd day of storage, fruits of mature stage, had more ascorbic acid content when packed in 3000 ppm KMnO₄ soaked paper i.e. 20.47 mg/100g whereas minimum was observed in control fruits i.e. 17.72 mg/100g. All the treatments of KMnO₄ were effective to retain the ascorbic acid and the effect of KMnO₄ increased with increasing concentrations of KMnO₄. Same effect was observed on 4th, 6th and 8th day of storage. Minimum ascorbic acid was observed in control fruits (3.17 mg/100g) after 10 days of storage in mature fruits whereas maximum was observed in fruits packed in 3000 ppm KMnO₄ (5.88 mg/100g) on same day of storage. In case of half ripe fruits, ascorbic acid decreased from 0 day (12.87 mg/100g) to 6th day (3.90 mg/100g) in control fruits whereas less decrease in ascorbic acid was observed in fruits packed with 3000 ppm KMnO₄ soaked paper i.e. from 0 day (12.87 mg/100g) to 6th day (4.37 mg/100g). In full ripe stage sapota fruits, ascorbic acid decreased from 9.08 mg/100g to 3.75 mg/100g on 0 day and 4th day respectively, in control fruits and from 9.08 mg/100g (0 day) to 3.52 mg/100g (4th day) in fruits packed with 3000 ppm

KMnO₄ soaked paper. Ascorbic acid was retained to higher extent with the application of KMnO₄. The effect increased with increasing concentrations of KMnO₄ on all period of storage. Fruits packed in KMnO₄ soaked paper had slow decrease in ascorbic acid content. This may be due to the reason that the various physiological processes might have been slowed down by absorbing ethylene with KMnO₄ application and affect increased with increase in KMnO₄ concentrations. These results are in conformity with the results of Ezzet *et al.* (2011) in Mango cv. Basennara, Tapre and Jain (2012) in Banana and Senjaliya *et al.* (2015) in Tomato from 15 mg/100g to 10 mg/100g.

Specific gravity: Data depicted in table 3 predicts that decrease in specific gravity was recorded in mature, half ripe and full ripe fruits of Sapota with increasing period of storage. Highest specific gravity was observed on 0 day i.e. 1.13 which decreased to 0.96 at the end of storage i.e. on 10th day of storage respectively, in mature fruits. In half ripe fruits, it decreased from 1.08 (0 day), 1.06 on 2nd day, and 1.03 on 4th day to 0.96 on the 6th day of storage. Maximum specific gravity (1.05) was recorded on the initial (0) day of storage and thereafter decreased to 1.01 on 2nd day and 0.97 on 4th day of storage respectively, in full ripe fruits. This may be due to the reason that more evaporative and transpirational losses which might have resulted in decrease in weight however volume might not have been decreased to same extent. So, the specific gravity of Sapota fruits decreased during the storage. Similar results of decrease in specific gravity from 1.05 to 0.95 after 8 days during storage have also been reported by Singh *et al.* (2012) in Mango and Kumari (2016) in Aonla. Maximum specific gravity (1.13) was observed on initial day of storage in all treatments. On 2nd day of storage, fruits of mature stage had maximum specific gravity when fruits were packed in 3000 ppm KMnO₄ soaked paper i.e. 1.12 whereas minimum specific gravity was observed in control fruits i.e. 1.09 on same day (2nd) of storage. All the treatments of KMnO₄ were effective to retain the specific gravity and the effect of KMnO₄ increased with increasing concentrations of KMnO₄ on all days of storage. Similar effect of KMnO₄ was observed in half and full ripe fruits. Fruits packed in KMnO₄ soaked paper had slow decrease in specific gravity content. This indicated that different treatments affected the mass and volume to equal extent as a result of which specific gravity remained same with different treatments. Similar results were also reported in Sapota fruit by Pawar *et al.* (2011) who studied decrease in specific gravity from 1.01 to 0.98 after 6 day of storage

Total phenols: Data depicted in table 4 predicts that decrease in total phenol content was recorded in mature, half ripe and full ripe fruits of sapota with increasing period of storage. Highest total phenol con-

tent was observed on 0 day i.e. 1.54 mg/100g which decreased to 1.15 mg/100g at the end of storage i.e. on 10th day of storage respectively, in mature fruits. In half ripe fruits, it decreased from 1.28 mg/100g (0 day) to 1.15 mg/100g on the 6th day of storage. Maximum total phenol content (1.17 mg/100g) was recorded on the initial (0) day of storage and thereafter decreased to 1.05 mg/100g on 4th day of storage respectively, in full ripe fruits. Phenolic content which are responsible for astringency of unripe fruits decreased may be either because of less synthesis or because of its higher oxidation. Decrease in total phenol content from 113 mg/100g to 72.2 mg/100g in different stages has also been reported by Miletic *et al.* (2012) in Plum and Kadarani *et al.* (2015) in Sugar apple fruits during storage. Maximum total phenol content (1.54 mg/100g) was observed on initial day of storage in all treatments. On 2nd day of storage, fruits of mature stage had maximum total phenol content when fruits were packed in 3000 ppm KMnO₄ soaked paper i.e. 1.51 mg/100g whereas minimum total phenol content was observed in control fruits i.e. 1.36 mg/100g on same day (2nd) of storage. All the treatments of KMnO₄ were effective to retain the total phenol and the effect of KMnO₄ increased with increasing concentrations of KMnO₄. Same effect was observed on 4th, 6th and 8th day of storage. Minimum total phenol was observed in control fruits (1.06 mg/100g) after 10th days of storage in mature fruits whereas maximum total phenol was observed in fruits packed in 3000 ppm KMnO₄ (1.26 mg/100g) on same day of storage. In case of half ripe fruits, total phenol decreased from 0 day (1.28 mg/100g) to 6th day (1.04 mg/100g) in control fruits whereas less decrease in total phenol was observed in fruits packed with 3000 ppm KMnO₄ soaked paper i.e. from 0 day (1.28 mg/100g) to 6th day (1.07 mg/100g). In full ripe stage sapota fruits, total phenol content decreased from 1.17 mg/100g to 1.05 mg/100g on 0 day and 4th day respectively, in control fruits and from 1.17 mg/100g (0 day) to 1.09 (4th day) in fruits packed with 3000 ppm KMnO₄ soaked paper. Total phenol was retained to higher extent with the application of KMnO₄. The effect increased with increasing concentrations of KMnO₄ on all period of storage. Fruits packed in KMnO₄ soaked paper had slow decrease in total phenol content. It might be due to delay in the process of ripening which might have maintained higher levels of phenols. Ali *et al.* (2015) in Apricot observed increase in phenol content from 210 mg/GAE 100g to 450 mg/GAE 100g and then decrease to 350 mg/GAE 100g.

Lipid peroxidation: The malondialdehyde (MDA) content which is an index of lipid peroxidation increased in all treatments and all stages during storage as presented in table 5. In biological system, lipid peroxidation generates a number of degradation products, such as MDA, which has been found to be an important cause of cell membrane destruction and cell

damage (Hodges *et al.*, 1999). Minimum MDA content was observed on 0 day i.e. 2.28 nmol which increased to 3.92 nmol at the end of storage i.e. on 10th day of storage respectively, in mature fruits. In half ripe fruits, it increased from 2.72 nmol(0 day) to 4.18 nmol on the 6th day of storage. Minimum MDA content (3.38 nmol) was recorded on the initial (0) day of storage and thereafter increased to 4.25 nmol on 4th day of storage respectively, in full ripe fruits. The increase in MDA content during ripening indicates increased production of reactive oxygen species during ripening thereby causing disruption of the membrane integrity of the fruit which in turn might have led to softening of fruit. Similar observations have been reported by Hu *et al.* (2011) in Pineapple and Kumar (2009) in Ber and. Who reported change in MDA content from 2.92 to 2.22 n mol during storage. Minimum MDA content (2.28 nmol) was observed on initial day of storage in all treatments. On 2nd day of storage, fruits of mature stage had minimum MDA content when fruits were packed in 3000 ppm KMnO₄ soaked paper i.e. 2.47 nmol whereas maximum MDA content was observed in control fruits i.e. 2.58 nmol on same day (2nd) of storage. All the treatments of KMnO₄ were effective to check the MDA content and the effect of KMnO₄ increased with increasing concentrations of KMnO₄. Same effect was observed on 4th, 6th and 8th day of storage. Maximum MDA was observed in control fruits (4.35 nmol) after 10th days of storage in mature fruits whereas minimum MDA was observed in fruits packed in 3000 ppm KMnO₄ (3.60 nmol) on same day of storage. In case of half ripe fruits, MDA increased from 0 day (2.72 nmol) to 6th day (4.46 nmol) in control fruits whereas less increase in MDA was observed in fruits packed with 3000 ppm KMnO₄ soaked paper i.e. from 0 day (2.72 nmol) to 6th day (3.98 nmol). In full ripe stage sapota fruits, MDA content increased from 3.38 nmol to 4.51 nmol on 0 day and 4th day respectively, in control fruits and from 3.38 nmol (0 day) to 3.97 nmol (4th day) in fruits packed with 3000 ppm KMnO₄ soaked paper. MDA content was decreased with the application of KMnO₄. The effect increased with increasing concentrations of KMnO₄ on all period of storage. Fruits packed in KMnO₄ soaked paper had slow increase in MDA content. It might be due to delay in ripening of sapota fruit and lower membrane deterioration. These observations are in agreement with those obtained by Wang (2014) in Hawthorns and Mondal *et al.* (2009) in Guava. Who observed increase in MDA content from 2.62 nmol to 5.11 nmol during storage.

Conclusion

The present study concluded that KMnO₄ was effective to reduce the loss in weight in all the three ripening stages and KMnO₄@ 3000 ppm was more effective. Specific gravity, ascorbic acid, total phenols decreased with increasing period of storage in all three ripening

stages whereas malondialdehyde content increased with increasing period of storage. At the end of storage maximum ascorbic acid (5.88 mg/100g) which is a strong antioxidant retained in mature fruits packed in KMnO₄@ 3000 ppm soaked paper. Total phenols which impart the characteristics flavour to fruits also retained in KMnO₄ treated fruits in all three stages and maximum in mature fruits (1.26 mg/g) packed in KMnO₄@ 3000 ppm soaked paper. Hence, all the concentrations of KMnO₄ was effective to increase the shelf life of fruits, as a results of which far off transportation of sapota become possible.

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