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Effects of ethanol-extracted propolis on the storage of Roomy Red grapes

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Abstract

The effect of ethanol extracted propolis (EEP) on storage of Red Roomy grapes under low temperature was investigated during 2008 and 2009 seasons. Dipping the clusters for 30 mints in EEP at 0 to 5% then dried with air and stored in especial plates at 1-2°C and 90±5% relative humidity resulted in the following main results:

All ethanol extracted propolis at 1 to 5% significantly reduced the percentages of the undesirable berries and berry weight loss compared to untreated one (control) during the two experimental seasons.

- Treatments with either 4 or 5% EEP gained the best results, where it caused a significant decreases in undesirable berries percentage by (65.54 & 66.88%) and (63.07 & 63.73%) and berry weight loss percentage (60.08 & 63.68%) and (46.36 & 47.63%) compared to control in both seasons, respectively.
- All treatments with EEP failed to show any significant effects on berry chemical properties compared to control.

It could be concluded that treatment with 4% ethanol extracted propolis seemed to be the proper and ideal treatment to prolong cold storage of Red Roomy grapes without great reduction on berry quality because it achieved the lowest figures in fresh weight losses, decay % and total acidity. In addition improved the reducing sugars.

Key words: Propolis, Roomy Red, Grapes, Cold Storage

Introduction

The Grapes is considered one of the most important fruit crop in the world, for being of an excellent flavor, nice taste and high nutritional value. In Egypt, it occupies the second position after citrus regarding the cultivated area and the magnitude of fruit production. No doubt that process of handling and storage for local or export market is an important and of vital interest as well as fruit production and its quality.

In post harvest handling of fruits for market, high concentration of chemicals are used for the prevention of fungal decay. The result of uncontrolled and excessive use of chemicals negatively affects human health and the environment. In addition, chemical residual effects on fruit can cause serious problems for export (Ozdemir *et al*., 2005). Moreover, it is necessary to explore effective and eco-friendly, normally safe fungicide alternative against

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these pathogens.

Propolis, a sticky substance produced by honeybees from plants exudates. It has a strong antibacterial, antiviral and antifungal and has been used for pharmacological applications (Ozdemir *et al*., 2010). Propolis can limits the growth of microorganisms such as *Candida* species, *Penicillium digitatum, P. italicum* (Herrera *et al*., 2010) and (Yang *et al*., 2010). Regarding its antimicrobial properties propolis could extent the shelf life of citrus, apple and sweet cherry (Candir *et al*., 2009; Ren *et al*., 2010; Yang *et al*., 2010 and Ozdemir *et al*., 2010). Also there were very few *in vitro* and *in vivo* studies have been conducted against plant pathogenic microorganisms by using propolis. This research aimed to study the effect of ethanol extracted propolis (EEP) on the preservation of grapes under low temperature (1-2°C) for eight weeks, and determine the effects of (1%, 2%, 3%, 4% and 5% concentrations) of EEP on maintaining fruit quality during the storage period.

Materials and Methods

This investigation was carried out throughout two successive seasons 2008 and 2009 on Red Roomy grapes in Pomology Laboratory of Horticulture Department, Faculty of Agriculture, Assiut University.

Preparation of propolis extracts:

Propolis samples used in this work were collected from apiary of Faculty of Agriculture, Assiut University, by scraping the walls and frames of the hives. Propolis extracts prepared as follows: 100 grams of propolis were frozen to -18°C, cut in small pieces, and ground in a chilled mortar than added to 900 ml of 70% ethanol. The mixture was gradually heated in water bath for 24 hours at 70°C. The extract was filtered and kept in a refrigerator unit use (Boeru V; Derevici, A ,1978). The amount of dissolved principles was assessed by weigh difference the 1%, 2%, 3% and 5%. Propolis extracts were prepared by making a dilution of the 10% propolis solution with water in the required preparation.

Plant material:

Clusters of (Roomy Red) cv. were harvested at the commercial maturity from the orchard of the Faculty of Agriculture, Assiut University. Such clusters free from infection with diseases and/or insects damage. Then these clusters were subjected to the following propolis treatments:

(1) Dipping in water (control).
(2) Dipping for 30 mints in 1% EEP (ethanol extracted propolis).
(3) Dipping for 30 mints in 2% EEP.
(4) Dipping for 30 mints in 3% EEP.
(5) Dipping for 30 mints in 4% EEP.
(6) Dipping for 30 mints in 5% EEP

Each treatment contained 3 replicates with six clusters for each, after drying the grapes with air (for one hour), they placed in plates covered with perforated plastic and stored at 1-2°C and 90$\pm$5% relative humidity for 8

weeks. Representative samples of each replicate were taken biweekly during storage period until the percentage of decay reached 50%. This experiment was arranged in a split plot design including six treatments and five periods with three replications (six clusters each). Physical and chemical properties were estimated biweekly as following:

(A) Physical characteristics:

1- Weight loss %:

This character was determined by weighing labeled two clusters from each replicate. Percentage of weight loss was calculated by determination the progressive reduction in cluster weight during storage period relative to the original fresh weight at the beginning of storage.

2-Undesirable fruits percentage:

Calculated by dividing the number of undesirable berries by the total number of berries according to the following equation:

$$\text{Berries decay \%} = \frac{\text{The number of decayed berries}}{\text{The total number of berries}} \times 100$$

(B) Chemical characteristics:

For estimating changes in the chemical properties during the cold storage conditions in both seasons. Representative samples of 25 berries from each replicate were randomizly selected biweekly intervals from the beginning until the end of storage period.

1 –Total soluble solids(TSS %):

TSS% in fruit juice was determined by using a hand refractometer.

2- Total acids %:

Total acidity % in berry juice was determined by titrating it against 0.1 N NaOH with phenol phethalin as an indicator and calculated as gram of tartaric acid as described in the A.O.A.C. (1995).

3- TSS/Acid ratio:

These values were calculated by dividing the percentage of total soluble solids (TSS %) on the total acid percentage in grape juice.

4- Sugar contents:

Reducing sugars contents percentage in grape juice were determined according to A.O.A.C. (1995).

All data obtained throughout this study were tabulated and statistically analysed, according to method described by Gomez and Gomez (1984) & Snedecor and Cochran (1990) and using L.S.D. test at 5% to recognize the significance of the differences among various treatments means.

Results and Discussion

Undesirable berries percentage:

Data presented in Tables (1 & 2) show the effect of ethanol extracted propolis (EEP) application on undesirable berries percentage of Roomy Red grapes during the cold storage in 2008 and 2009 seasons. It was obvious from the data that results took similar trend during the two studied seasons.

In response of propolis application, it was apparent that all treatments significantly reduced the undesirable berries percentage during cooling storage for

eight weeks compared with untreated ones (control). Propolis at 5% gave the least undesirable berries percentage (as an average of 8.64 & 9.39%) in the two studied seasons, respectively.

No significant differences due to whatever 4% or 5% propolis treatment. Thus the money-wise evaluation of these treatments was in favour of 4% ethanol extracted propolis which recorded (8.99 & 9.56%) of undesirable berries percentage compared to (26.09 & 25.89%) in untreated clusters during the two studied seasons, respectively. Therefore, the decrement percentage of undesirable berries due to 4 and 5% propolis treated under untreated ones were attained (as an average of 65.54 & 66.88%) and (63.07 & 63.73) in 2008 and 2009 seasons, respectively. Such results may be due to antifungal activity of propolis constituents that reduced the incidence of berry drop, berry rat and total spoilage during storage.

Moreover, data in prementioned tables showed that undesirable berries percentage significantly increased by extending cooling storage duration. The undesirable berries percentage slightly increased and gradually from the beginning of cold storage till the 6^{th} week attaining (16.68 & 17.55%) in the two studied seasons, respectively. Prolonging storage period thereafter, highly undesirable berries occurred attaining (27.14 & 27.34%) and the increasing percentage compared to 6^{th} week were (62.71 and 55.78%) in both seasons, respectively.

According to interaction effects, data in tables (1 & 2) indicated that all combination after 2 weeks induced a significant decrease in such trait compared with untreated one. Moreover, using either 4 or 5% propolis recorded the lowest values (19.48 & 18.83%) and (19.68 & 18.59%) compared to other treatments in both seasons, respectively. Thus, the decrement percentage were attained (59.21 &60.57%) and (56.60 & 58.96%) due to 4 or 5% propolis compared to untreated one in the first and second studied seasons, respectively.

These findings are in agreement with those obtained by Candir *et al*. (2009) who found that treatment with 5% EEP was effective in preventing fungal decay in cherries during storage at 0°C for 4 weeks. Also, Ozdmir *et al*. (2010) reported that treatment with 5% EEP produced grapes with a significantly reduced incidence of fungal decay. Also, Yang *et al*. (2010) reported that propolis ethyl-acetate extract (PEAE) could reduced decay caused by both *Penicillium digitatum* and *P. italicum* in wound-inoculated citrus fruits and naturally infected fruits. These results may be due to the antifungal activity of propolis constituents, such as caffeic acid, Peterostilbene and Sakuranetin (Ghisalberti, 1979).

Table 1: Effect of propolis (EEP) on undesirable berries percentage of Roomy Red grapes under cold storage during 2008 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
2	0.87	0.61	0.70	0.68	0.52	1.89	0.85
4	7.78	9.62	7.97	4.99	5.16	22.57	9.68
6	16.77	15.65	14.66	10.82	10.06	32.15	16.68
8	28.38	24.32	23.27	19.48	18.83	47.76	27.14
Mean	**13.45**	**12.76**	**11.65**	**8.99**	**8.64**	**26.09**	

LSD 5%: A= 2.14 B= 2.33AB= 4.69

A: Dipping clusters in ethanol extracted propolis (EEP)

B: Cold storage period.

AB: Interaction between AxB

Table 2: Effect of propolis (EEP) on undesirable berries percentage of Roomy Red grapes under cold storage during 2009 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
2	2.88	2.25	1.98	1.59	1.28	3.09	2.18
4	9.17	9.81	7.27	5.90	6.76	22.30	10.19
6	17.16	16.48	16.83	11.07	10.41	32.86	17.55
8	28.20	26.95	25.31	19.66	18.59	45.30	27.34
Mean	**14.35**	**13.87**	**12.58**	**9.56**	**9.39**	**25.89**	

LSD 5%: A= 3.15 B= 3.41AB= 6.68

(2) Berry weight loss %:

Data illustrated in tables (3 & 4) showed that all treatments significantly decreased the berry weight loss percentage during cooling storage for eight weeks compared to control. There were no significant differences between 4% and 5% ethanol extracted propolis (EEP) treatments. Using 5% EEP has the best results, which gave the least values of berry weight loss percentage (1.88 & 2.44%) in both studied seasons, respectively. The corresponding decrement percentage of berry weight loss due such treatment under control attained (61.16 & 52.53%), respectively. These results may be due to making a thin film of propolis (wax) surrounding the berry peel, which induced a modification of microclimatic fruit, then reduce of water loss from the berries.

These findings are in agreement with Kaska and Dundar (1992), Pekmezci *et al*. (1995), Hagenmaier and Baker (1996), Ozdemir and Dundar (1999) and Ozdemir and Dundar (2001), they reported that weight loss was lowering with propolis treatments. Also, Ozdmir *et al*. (2010), who found that EEP

treatments reduced grape fruits weight loss % compared with controls. Ren, Yan *et al*. (2010) found that 8% concentration of propolis could effectively inhibit the water loss of apple fruits during cold storage at 0-1°C for 180 days. On the other hand, Candir *et al*. (2009) showed that EEP treatment at 1% & 5% and 10% concentrations had no incidence effect on sweet cherry fruits weight loss during cold storage at 0°C for 4 weeks.

Data in the previously tables indicated that the weight loss of berries slightly increased in a gradual manner from the beginning of cold storage till 6th week attained (3.08 & 4.20%) in both studied seasons, respectively. Prolonging storage period thereafter, (8th week), highly weight loss occurred attaining (5.72 & 7.71%) with increment percentage (85.71 & 83.71%) compared to 6th weeks period in both seasons, respectively.

These findings may be due to loss of moisture from the berries (Ozdmir *et al*., 2010). Water loss can be one of the main causes of deterioration, since it is not only quantitative losses, but also causes losses in appearance, due to willing and shriveling and nutritional quality (Kader, 1986).

Also, the obtained data indicated that all treatments reduced the weight loss of berries compared with control in any time during cold storage. Moreover, such reduction was significant when 4% or 5% propolis treatment was applied. After eight weeks, treatment with 4% or 5% recorded the least weight loss values (3.88 & 3.53%) and (5.89 & 5.75%) compared with (9.72 & 10.98%) for untreated one. Hence, the decrement percentage of weight loss % attained (60.08 & 63.68%) and (46.36 & 47.63%) in the first and second seasons, respectively.

Table 3: Effect of propolis on berry weight loss % of Roomy Red grapes under cold storage during 2008 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
2	0.75	0.90	0.86	0.76	0.69	1.16	0.85
4	2.50	2.34	2.41	1.58	1.41	3.73	2.31
6	3.41	3.15	3.13	2.14	1.88	4.76	3.08
8	6.92	5.28	4.96	3.88	3.53	9.72	5.72
Mean	**3.40**	**2.92**	**2.84**	**2.06**	**1.88**	**4.84**	

LSD 5%: A= 0.94 B= 1.05AB= 2.09

Table 4: Effect of propolis on berry weight loss % of Roomy Red grapes under cold storage during 2009 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
2	0.56	0.51	0.43	0.32	0.28	0.81	0.49
4	3.22	3.18	3.05	1.32	1.18	3.34	2.55
6	4.92	4.90	4.67	2.71	2.56	5.43	4.20
8	8.21	7.75	7.68	5.89	5.75	10.98	7.71
Mean	**4.23**	**4.09**	**3.96**	**2.56**	**2.44**	**5.14**	

LSD 5%: A= 0.75 B= 0.82AB= 1.61

(3) Berry chemical properties:

It is clear from the data in Tables (5 to 12) that all treatments with ethanol extracted propolis failed to show any significant effects on berry quality compared to untreated ones.

Accordingly, data indicated that berry quality in terms, total soluble solids, reducing sugars and total acidity significant increased during storage duration period up to the 8th week recording (18.34 & 18.17), (14.04 &14.06%) and (0.425 & 0.422%) for total soluble solids, reducing sugars and total acidity in both seasons, respectively. These results could be due to loosing amount of berry organic acids in metabolism activities as well as due to concentration since water loss. On the other hand, prolonging cooling storage for eight weeks induce a gradually decrease of total soluble solids/acid ratio. Such findings could be due to increase the total acidity percentage as prolong the storage period.

These results are in contrast with Ren Yan *et al*. (2010) as they showed that treatment with 8% propolis could effectively delay the degeneration of total soluble solids percentage comparing with control, during cold storage at 0-1°C for 180 days. However, the effect of EEP at 1%, 5% and 10% concentrating on the TSS content were not significant during 6 months of storage at 8°C (Ozdemir *et al*., 2010).

The interaction between treatments and cold storage period was significant for all studied chemical properties in both studied seasons. The highest values of total soluble solids (19.06 & 18.87) and total acidity (0.442 & 0.470) came from untreated ones (control), however, the highest values of reducing sugar (14.29 & 14.36) were obtained from 4% propolis at the end of storage period compared with other interaction treatments at such storage period in both seasons, respectively. On other hand, least values of total soluble solids/acid ratio (42.93 & 41.48) were recorded on untreated ones compared to other interaction treatments in both seasons, respectively.

According to the previous results, it could be concluded that treatment with 4% ethanol extracted propolis seemed to be the proper and ideal treatment to prolong cold storage of Red Roomy grapes without great reduction on berry quality because it achieved the lowest, decay % and total acidity. In addition improving the reducing sugars.

Table 5:Effect of propolis on Total Soluble Solids percentage (TSS%) of Roomy Red grapes under cold storage during 2008 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	15.87	16.10	16.27	16.00	16.20	16.07	16.09
2	16.40	16.40	17.13	16.67	17.07	16.53	16.70
4	17.73	17.00	17.73	17.22	17.62	17.30	17.43
6	18.00	17.30	18.00	17.80	17.80	18.10	17.83
8	18.67	17.80	18.10	18.20	18.20	19.06	18.34
Mean	**17.33**	**16.92**	**17.45**	**17.18**	**17.40**	**17.40**	

LSD 5%: A= N.S. B= 0.66AB= 1.62

Table 6: Effect of propolis on Total Soluble Solids percentage (TSS%) of Roomy Red grapes under cold storage during 2009 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	16.00	16.00	16.27	16.30	16.63	16.08	16.21
2	16.80	17.00	17.00	16.85	17.10	16.87	16.94
4	17.00	17.10	17.30	17.10	17.10	17.26	17.14
6	17.35	17.20	17.30	17.60	17.50	18.00	17.49
8	18.10	17.70	18.00	18.20	18.10	18.87	18.17
Mean	**17.08**	**17.00**	**17.17**	**17.21**	**17.29**	**17.42**	

LSD 5%: A= N.S. B= 0.74AB= 1.66

Table 7: Effect of propolis on Total acidity of Roomy Red grapes under cold storage during 2008 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	0.210	0.215	0.200	0.210	0.205	0.210	0.208
2	0.240	0.260	0.270	0.240	0.245	0.255	0.252
4	0.308	0.293	0.318	0.315	0.327	0.328	0.315
6	0.348	0.320	0.338	0.320	0.340	0.365	0.338
8	0.412	0.408	0.400	0.400	0.402	0.442	0.425
Mean	**0.304**	**0.299**	**0.305**	**0.296**	**0.304**	**0.320**	

LSD 5%: A= N.S. B= 0.034 AB= 0.075

Table 8: Effect of propolis on Total acidity of Roomy Red grapes under cold storage during 2009 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	0.215	0.215	0.225	0.220	0.233	0.225	0.223
2	0.255	0.260	0.246	0.240	0.248	0.265	0.252
4	0.263	0.298	0.287	0.305	0.315	0.309	0.296
6	0.340	0.315	0.320	0.350	0.358	0.380	0.344
8	0.425	0.405	0.420	0.410	0.407	0.470	0.422
Mean	**0.300**	**0.300**	**0.299**	**0.305**	**0.311**	**0.330**	

LSD 5%: A= N.S. B= 0.044 AB= 0.098

Table 9: Effect of propolis on TSS/acidity of Roomy Red grapes under cold storage during 2008 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	75.58	74.89	73.98	76.22	78.83	76.45	75.98
2	68.33	63.10	63.45	68.56	69.70	64.65	66.29
4	57.58	58.12	55.25	54.86	53.98	52.71	55.41
6	51.72	54.11	53.25	55.61	52.36	49.62	52.78
8	45.32	43.65	45.28	45.50	45.34	42.93	44.76
Mean	**59.71**	**58.39**	**58.24**	**60.24**	**60.04**	**57.32**	

LSD 5%: A= N.S. B= 2.89AB= 6.52

Table 10: Effect of propolis on TSS/acidity of Roomy Red grapes under cold storage during 2009 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	74.00	74.12	71.80	74.10	71.45	72.78	73.04
2	65.33	65.03	68.33	69.91	68.58	64.95	67.02
4	64.31	57.51	60.00	56.23	54.44	56.95	58.18
6	50.53	54.22	53.60	50.46	49.18	48.55	51.09
8	42.10	43.60	42.62	44.45	44.58	41.48	43.15
Mean	**59.21**	**58.90**	**59.22**	**59.03**	**57.66**	**56.94**	

LSD 5%: A= N.S. B= 2.43AB= 5.48

Table 11: Effect of propolis on reducing sugars of Roomy Red grapes under cold storage during 2008 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	12.09	12.26	12.50	12.48	12.59	12.28	12.37
2	12.60	12.58	13.21	13.10	13.18	12.73	12.90
4	13.53	13.09	13.63	13.61	13.86	13.50	13.54
6	13.68	13.65	13.76	14.05	13.96	13.60	13.78
8	13.95	13.93	13.98	14.29	14.14	13.90	14.03
Mean	**13.17**	**13.10**	**13.42**	**13.51**	**13.55**	**13.20**	

LSD 5%: A= N.S. B= 0.58AB= 1.30

Table 12: Effect of propolis on reducing sugars of Roomy Red grapes under cold storage during 2009 season.

EEP Conc. (A) / Week (B)	1%	2%	3%	4%	5%	Cont.	Mean
0	12.36	12.43	12.61	12.58	12.74	12.38	12.52
2	13.03	13.16	13.23	13.30	13.51	12.96	13.20
4	13.19	13.28	13.46	13.56	13.64	13.19	13.39
6	13.58	13.64	13.54	13.89	13.81	13.63	13.68
8	13.92	13.98	13.97	14.36	14.30	13.85	14.06
Mean	**13.22**	**13.30**	**13.36**	**13.54**	**13.60**	**13.20**	

LSD 5%: A= N.S. B= 0.66AB= 1.48

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تأثير المستخلص الكحولي لصمغ النحل (البروبوليس) علي تخزين ثمار العنب الرومي الأحمر

علاء عبدالجابر بدوى مسعود ، ابتسام فتحى محمود
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تحت دراسة تأثير المستخلص الكحولي لصمغ النحل (البروبوليس) علي تخزين عناقيد العنب الرومي خلال موسمي 2008 و 2009 وذلك في معامل قسم البساتين (فاكهة) – كلية الزراعة – جامعة أسيوط حيث تم نقع العناقيد المعدة للتخزين في تركيزات صفر ، 1 ، 2 ، 3 ، 4 ، 5% لمدة نصف ساعة ثم يجري التخزين المبرد علي درجة 1-2°م .

وقد أوضحت النتائج:

- أدت جميع المعاملات بالمستخلص إلي خفض النسبة المئوية لحبات العنب التالفة خلال فترة التخزين مقارنة بالحبات الغير معاملة خلال موسمي الدراسة.
- كانت أفضل المعاملات هي المعاملة بتركيزات 4 و 5% حيث أدت إلي نقص معنوي في نسبة الثمار التالفة (65.54 ، 66.88) و (63.07 ، 63.73%) وكذلك نقص معنوي في النسبة المئوية للفقد في الوزن (60.08، 63.68) و (46.36 ، 47.63%) مقارنة بالثمار الغير معاملة خلال موسمي الدراسة علي التوالي.
- لم تظهر المعاملات أي تأثيرات علي خصائص الحبات الكيميائية.

من نتائج هذه الدراسة يمكن التوصية باستخدام المستخلص الكحولي لصمغ النحل بتركيز 4% وذلك لإطالة فترة تخزين ثمار العنب الرومي حيث تؤدي هذه المعاملة لتقليل نقص الوزن وتلف الثمار بالإضافة إلي تقليل نسبة الحموضة وتحسين نسبة السكريات.