



ENHANCING STORAGE EFFICIENCY OF POMEGRANATE FRUITS USING ALOE VERA GEL AND SOME NATURAL OILS

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Abstract

Wonderful pomegranate fruits were coated with 10, 15 or 20% concentrations of *Aloe vera* gel (*Aloe barbadensis* Miller) combined with almond or coconut oil to study their effects on physical and chemical fruit properties when the fruits were stored at 5°C and 85 - 90% RH for a total storage period of 45 days and compared with the untreated fruits (control). The obtained results show that, it could be concluded that treating pomegranate fruits with aloe vera gel combined with almond or coconut oils had a positive effect on prolonging fruit storage with good properties along the storage periods comparing with the untreated fruits (control). In this concern, using aloe vera gel at 10 or 15% was more effective than 20% concentration especially when combined with almond or coconut oil in keeping fruits in good quality along the storage periods.

Key words: Pomegranate, *Aloe vera* gel, almond oil, coconut oil, fruit properties, storage.

Introduction

Pomegranate (*Punica granatum*) is belonging to family Punicacea and widely cultivated in Middle East, the Mediterranean countries and the United States (Holland *et al.*, 2009). Pomegranate is an important fruit crop, it is considered one of the promising exportation fruits in Egypt in the last years (Abd-elghany *et al.*, 2012). The fruit consumption is growing due in part to its unique sensory and nutritional properties coupled with medicinal benefits attributed to high content of phytonutrients and antioxidant properties (Hassan *et al.*, 2012). The high antioxidant activities of pomegranate fruit are attributed to high levels of polyphenolic compounds, which act as good free radical scavengers (Fawole and Opara, 2012).

Wonderful pomegranate is late cultivar with high yield, large fruit, rich red aril, high juice, and good palatability (Palou *et al.*, 2007). Wonderful is currently one of the most desired planted pomegranate cultivars in Egypt since it offers best balance combination yield and quality (Abd-elghany *et al.*, 2012). The pomegranate cv. Wonderful is also known for its health benefits that may help in the prevention of heart disease, cancer and problems associated with aging (Anoun, 2004).

Edible coating is considered one of the most accepted methods for prolonging the commercial shelf life of fruits and is an innovative method for controlling fruits quality through minimizing microbial postharvest losses. Moreover, the application of coating materials affects on the nutritional composition and fresh appearance of fruits (ÖZ and Eker, 2017). There are a number of materials that are commonly used as edible coatings or films are lipids, resins, polysaccharides, proteins and others of natural products. The most important benefits of edible coatings are a decrease in the synthetic packaging waste and a contribution to food health and safety while meeting the environmental requirements (Garcia *et al.*, 2010; Kamboj and Kaur, 2018).

The natural products are safe and environmentally friendly alternative to synthetic preservatives (Serrano *et al.*,

2006). *Aloe vera* gel has been proven one of the best edible and biologically safe preservative coatings for different types of foods because of its film-forming properties, antimicrobial actions, and biodegradability and biochemical properties. It is composed mainly of polysaccharides and acts as a natural barrier to moisture and oxygen, which are the main agents of deterioration of fruits and vegetables (Misir *et al.*, 2014). Moreover, *Aloe vera* is a rich source of many chemical compounds. *Aloe vera* gel contains 75 nutrients and 200 active compounds including sugar, anthraquinones, saponins, vitamins, enzymes, minerals, lignin, salicylic acid and amino acids (Dureja *et al.*, 2005). *Aloe vera* gel-based edible coatings have been shown to prevent loss of moisture and firmness, control respiratory rate and maturation development, prolonging storage/shelf-life, delay oxidative browning and reduce microorganism proliferation in pomegranate arils (Martínez-Romero *et al.*, 2013)

On the other hand, addition of oils to biofilms causes some changes in the physical and mechanical properties provides to the films an efficient antifungal capacity and protect food from contamination of pathogenic bacteria during storage (Ma *et al.*, 2016). Accordingly, almond oil has been used as natural edible coating alone or in combination with other ingredients to coat fruits for its ability to prevent water loss, reduce respiration, softening of arils, weight loss and browning index, loss of vitamin C, loss of anthocyanin and delayed microbial decay thereby extending shelf life and to improve appearance by generating a shiny product in fruits (Singh *et al.*, 2017).

Coconut oil as edible coating of fruits has gaining interest for its anti-senescence property by controlling respiration rate, transpiration rate and binding of the ethylene biosynthesis process. Also coconut oil coating closed the opening of stomata and lenticels thereby, reducing the transpiration and respiration rate (Nasrin *et al.*, 2018) and also reduce microbial activity due to contains lauric acid which adds antimicrobial property to the coatings and impart moisture barrier to the hydrophilic coatings (Kamboj and Kaur, 2018).

The aim of this study, is to evaluate the effect of postharvest edible coatings by *Aloe vera* gel, coconut oil and almond oil for maintaining fruit quality attributes and extend the storage period of Wonderful pomegranate fruits.

Materials and Methods

Fruits

Pomegranates fruits cv. Wonderful (*Punica granatum* L.) were obtained from experimental station of National Research Centre, at Al-Nobaria district, Al-Behera governorate, Egypt. Fruits were harvested at maturity stage from seven years old trees that were similar in growth vigor and subjected to the common horticultural treatments. Mature fruits were harvested to be similar as possible in color and size, and free of any noticeable pathological or mechanical injuries. Fruits were instantly packed and transported to the laboratory, all fruits washed by tap water and air dried. The initial quality measurements were determined. The shown results are average for two successive seasons 2017 and 2018.

Treatments

Pomegranate fruits were coated with different concentrations of *Aloe vera* gel (*Aloe barbadensis* Miller) 10%, 15% and 20% combined with different form of natural oils, which were prepared from the oil extraction unit of National Research Centre such as almond (*Prunus dulcis* Mill) seed oil and coconut (*Cocos nucifera*) fruit oil as 0.1% concentration. Glycerol 5% was then added to the *Aloe vera* gel with oils mixture.

Aloe vera Coating Preparation

Fresh *A. vera* gel was prepared according to previous reports (Navarro *et al.*, 2011). Briefly, for each leaf the spikes along the margins were removed before longitudinally slicing to separate the rind from the inner leaf gel. The gel fillets were crushed to yield a mucilaginous gel which was filtered to discard the fibrous fraction. The gel was diluted with distilled water for the *A. vera* 10%, 15% and 20% (v/v) treatments.

Almond and Coconut oils Preparation

Oils prepared by dissolved 0.1ml oil in 100 ml of distilled water to 0.1% concentration, Glycerol was prepared by dissolved 5 ml glycerol in 100 ml of distilled water to 5 % concentration, and used in the further experiment.

After postharvest coating treatments, fruits were air dried and packed in corrugated cardboard boxes and placed in a cold storage room at 5°C and 85 - 90% RH for a total storage period of 45 days and compared with the untreated fruits (control). Three replicates for each treatment and sampling time (15 days) were used and each replicate consists of three fruits. Fruit quality measurements were assessed after storage at 5°C in each sampling date.

Postharvest Determinations

Physical Properties Assessments:

Fruit weight loss (FWL): Fruits were weighed at the beginning and after an interval of 7 days for a period of 45 days storage. The fruit weight loss percent was calculated by standard procedure as the following equation. Fruit weight loss % = wt. of 1st interval – wt. of 2nd interval x100 / wt. of first interval.

Whole fruit weight (gm) and fruit volume (cm³) were measured. Fruits were carefully opened to avoid damaging arils. The arils were separated from pericarp fractions, the total aril weight per fruit were obtained. Arils were then manually juiced and residual pulp carefully cleaned from the seeds, aril juice weight(gm) and volume (cm³) were measured.

Fruit Firmness:

Fruit firmness was determined using Ametek pressure tester. Firmness of 3 fruits from each replicate was measured at two opposite points on the equator of each fruit. Results were calculated as Ib/inch² (AOAC., 1990).

Soluble solids content (SSC, %): SSC content was measured using a T/C hand refractometer Instrone, Brix-readings 0 - 30 ranges (Model 10430, Bausch and Lomb Co. Calif., USA) and express in percentage.

Chemical Properties Assessments

Titrateable acidity (TA, %): total acidity content (expressed as citric acid) was determined by titrating 5 ml juice with 0.1N sodium hydroxide using phenolphthalein as an indicator. Ascorbic acid content (VC) was measured using 2, 6 dichlorophenol indophenols' method described by A.O.A.C. (1990).

Total anthocyanins content (TAC): Anthocyanin of arils juice (mg/100g arils) was measured colorimetrically at 535 nm in arils juice according to the methods of Fuleki and Francis (1968)

Total antioxidant content (TAC): Antioxidant activity in the pomegranate arils was assessed by using the free radical DPPH method (Bond and Michel, 1997).

Pectinase Activity (PA):

Sample of 0.5 ml of supernatant enzyme extraction were used and mixed in acetate buffer then incubated at 45 °C for 10 min for pectinase. The reaction was stopped with 3 ml of 3, 5-dinitrosalicylic acid reagent, the color was obtained after heating for 10 min., and measured at wavelength of 570 nm and expressed as one unit of pectinase activity liberates 1 Mmol D-galactouronic acid in milliliter per min (Miller, 1959).

Statistical Analysis

The design for this experiment was a completely randomized design (CRD) with three replications. The collected data on various parameters were statistically analyzed using variance (ANOVA) procedure of MSTATC program. Treatments means were compared by Duncan's multiple range tests at 5% level of probability in the average of two seasons of study (Steel and Torrie, 1980).

Results and Discussion

Fruit Physical Properties

The effect of *Aloe vera* gel concentrations either with almond or coconut oil on fruit physical properties of pomegranate were studied as follows.

Fruit weight results in Table (1) show that fruit weight was gradually decreased as the days of storage increased. This means that the fruit weight was at low value at the end of the storage period (after 45 days) comparing with the beginning of the experiment. This was true for all treatments

under investigation including the control. On the other hand, aloe vera gel at 15% + coconut oil treatment recorded the heaviest fruit (332 gm) after 45 days of storage followed in decreasing order by aloe vera gel at 15% + almond oil which recorded 328 gm. The lightest fruit was recorded when the fruits treated with 10% *Aloe vera* gel + almond oil, while the other treatments gave intermediate values.

Concerning the fruit volume, the obtained results in Table (2) took somewhat the same line of the fruit weight, since the fruit volume was reduced gradually along the storage period. The treatment of *Aloe vera* gel at 15% + coconut oil recorded the highest value at the end of the storage period, while aloe vera gel at 10% + almond oil gave the lowest volume of the fruits.

As for fruit weight loss percentage, it is clear from the results presented in Table (3) that the weight loss was increased due to the increasing of storage period. This observation was for all treatments. However, at the end of the experiment, the highest value of fruit weight loss (42.9%) was recorded with the untreated fruits (control) followed without significance by the fruits treated with *Aloe vera* gel at 15% + almond oil (42.1%). The lowest value of the weight loss was recorded by the treatment of 20% *Aloe vera* gel + coconut oil (28.15%) followed without significance by 10% *Aloe vera* gel + almond oil (29.29%).

Regarding fruit firmness (Table 4), it is observed that the value of fruit firmness was decreased at the end of the storage period compared with the beginning of the experiment. This observation was detected for all treatments. However, the untreated fruits (control) kept the fruit firmness at higher level followed by treating fruits with 10% *Aloe vera* gel + almond oil comparing with the other treatments. The lowest fruit firmness at the end of the experiment was recorded due to *Aloe vera* gel at 10% + coconut oil.

As for juice weight, results presented in Table (5) show in general that there was a reducing in juice weight value among all treatments after 45 days of the storage comparing with zero day. On the other hand, using *Aloe vera* gel at 15% + almond achieved the highest value of juice weight followed in decreasing order by 10% *Aloe vera* gel + coconut oil. This means that these treatments are more effective in saving the juice weight without losses than the other treatments including the control.

The same trend of juice weight was detected with juice volume in Table (6), since there was a reduction in juice volume at the end of the storage period when compared with the zero day of the storage. This result was observed with all treatments under investigation including the control. However, it is observed that 10% *Aloe vera* gel + coconut oil treatment recorded the highest volume of juice after 45 days of storage followed by 20% *Aloe vera* + almond oil treatment. The untreated fruits gave the lowest value, while the other treatments recorded intermediate values.

As for seed weight, results in Table (7) show that seed weight tended to decrease during the storage period for all treatments under the experiment. The lowest seed weight was obtained due to 10% *Aloe vera* gel + almond oil, while, the highest seed weight was recorded due to *Aloe vera* gel at 15% + almond oil.

Fruit Chemical Properties

The effect of aloe vera gel either with aloe vera or coconut oil on total soluble solids percentage is presented in Table (8). The obtained results show in general that TSS% was gradually increased during the storage period with all treatments. The differences among the treatments were significant at the end of the storage period. In this concern, the highest TSS percentage was recorded by *Aloe vera* gel at 10% + coconut followed without significance by *Aloe vera* gel at 15% + almond oil or the control treatments. The other treatments recorded lower percentage without significance among them.

As for acidity, results in Table (9) show in general that acidity percentage was reduced along the storage period for all treatments, this means that at the end of the storage period (after 45 days) the acidity recorded the lowest value for each treatment. However, *Aloe vera* gel at 15% + almond oil and *Aloe vera* at 15% + coconut oil recorded lower acid percentages after 45 days of storage comparing with the other treatments. The highest acidity value was recorded due to *Aloe vera* gel at 10% + coconut oil followed without significance by *Aloe vera* gel at 20% + almond oil.

Regarding pectinase content in the fruit pulp, results in Table (10) clear that pectinase activity was increased gradually along the storage period and the highest value for each treatment was recorded when the storage reached the day 45. Among the treatments, the control recorded the highest significant pectinase U/g (12.56) comparing with the other treatments after 45 days of storage, while treating fruits with *Aloe vera* gel at 15% + almond oil recorded the lowest value (4.02 U/g) followed by *Aloe vera* gel at 20% + almond oil.

Concerning antioxidant activity in the fruit juice, the obtained results in Table (11) show that among all treatments, the antioxidant activity was gradually increased due to increasing the storage period. However, treatment of *Aloe vera* gel at 10% + almond oil recorded the highest value (75.7 µg/ml juice) at the end of the storage (after 45 days) followed in decreasing order and the differences lacked significance by *Aloe vera* gel at 10% + coconut oil, while the lowest value of antioxidant activity was recorded when the fruits treated by *Aloe vera* at 20% + almond oil. The other treatments gave intermediate values.

Discussion

It's clear from the obtained results that *Aloe vera* gel concentrations had a good effect on physical and chemical properties of pomegranate fruits that stored at 5°C and 85 – 90 RH for 45 days. The good effect of aloe vera could be due to that *Aloe vera* contains polysaccharides and acts as a natural barrier to moisture and oxygen, which are the main agents of deterioration of fruits and vegetables (Misir *et al.*, 2014). In this concern, *Aloe vera* gel have been shown to prevent loss of moisture and firmness, control respiratory rate and maturation development, prolonging storage/shelf-life, delay oxidative browning and reduce microorganism proliferation in pomegranate arils (Martínez-Romero *et al.*, 2013).

The effect of *Aloe vera* was improved by adding almond or coconut oils. In this respect, the effect of adding almond oil to *Aloe vera* may be due to its effect on preventing water loss, reducing respiration, softening of arils,

weight loss and browning index, loss of vitamin C, loss of anthocyanin and delaying microbial decay thereby extending shelf life and to improve appearance by generating a shiny product in fruits (Singh *et al.*, 2017).

On the other hand, the positive effect of adding coconut oil could be explained due to its anti-senescence property by controlling respiration rate, transpiration rate and binding of the ethylene biosynthesis process. Coconut oil coating closed the opening of stomata and lenticels thereby, reduced the transpiration and respiration rate (Nasrin *et al.*, 2018) and also reduce microbial activity due to containing of lauric acid which adds antimicrobial property to the coatings and impart

moisture barrier to the hydrophilic coatings (Kamboj and Kaur, 2018).

Conclusion

From the above mentioned results, it could be concluded that treating pomegranate fruits with *Aloe vera* gel combined with almond or coconut oils had a positive effect on prolonging fruit storage with good properties along the storage periods comparing with the untreated fruits (control). In this concern, using *Aloe vera* gel at 10 or 15% was more effective than 20% concentration especially when combined with almond or coconut oil in keeping fruits in good quality along the storage periods.

Table 1 : Fruit weight of pomegranate as affected by aloe vera gel and almond or coconut oil under storage conditions.

Fruit weight (g)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	363 c	363 c	363 c	363 c	363 c	363 c	363 c
	15	255 lm	373 b	385 a	385 d	355 d	299 j	327 hi
	30	245.3 n	347 e	214 n	339 f	349 e	283 k	324 l
	45	192 q	328 h	219 o	257 l	332 g	253 m	214 p

Table 2: Fruit volume of pomegranate as affected by aloe vera gel and almond or coconut oil under storage conditions.

Fruit volume (cm ³)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	468 a	468 a	468 a	468 a	468 a	468 a	468 a
	15	300 hi	425 abc	465 a	410 bcd	450 ab	340 fgh	380 cdef
	30	240 jk	372.5 def	450 ab	390 cde	425 abc	300 hi	360 efg
	45	200 k	316.7 ghi	325 gh	325 gh	400 cde	275 ij	225 k

Table 3: Weight loss of pomegranate fruits as affected by aloe vera gel and almond or coconut oil under storage conditions.

weight Loss (%)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	0 i	0 i	0 i	0 i	0 i	0 i	0 i
	15	12.64 h	14.72 fgh	15.75 f	13.84 fgh	12.35 h	13.17 gh	15.33 fg
	30	24.11 e	23.60 d	23.55 e	23.54 e	24.45 e	22.93 e	30.25 d
	45	29.29 d	42.10 a	40.75 ab	35.40 c	38.45 b	28.15 d	42.92 a

Table 4 : Fruit firmness of pomegranate as affected by aloe vera gel and almond or coconut oil under storage conditions.

Fruit firmness (lb/in ch ²)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	18 a	18 a	18 a	18 a	18 a	18 a	18 a
	15	10.65 cde	11.50 bc	10 def	11 bcd	12 b	11.90 bc	9.80 def
	30	9.65 efg	11.40 bc	7.50 ijk	8.25 hij	11.85 bc	8.70 fhij	9.45 efggh
	45	8.35 ghij	8 ij	6.25 kl	6.05 l	7.45 jk	7.60 ij	8.80 fghi

Table 5 : Juice weight of pomegranate fruits as affected by aloe vera gel and almond or coconut oil under storage conditions.

Juice weight (gm)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	191 a	191 a	191 a	191 a	191 a	191 a	191 a
	15	177 b	180 b	163 c	151e	132 g	122 h	157 d
	30	102 k	117 i	148 e	133 g	130 g	112 j	150 e
	45	89.67 l	144 f	91 l	133 g	111 j	99 k	78 m

Table 6: Juice volume of pomegranate fruits as affected by aloe vera gel and almond or coconut oil under storage conditions.

Juice Volume (cm ³)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	181 a	181 a	181 a	181 a	181 a	181 a	181 a
	15	158 b	180 a	154 bc	147 cd	137 e	141 d	150 c
	30	112 gh	157 b	170 e	142 d	114 g	116 g	125 f
	45	98.5 i	111 h	117 g	125 f	110 h	108 h	87 i

Table 7 : Seed weight of pomegranate fruits as affected by aloe vera gel and almond or coconut oil under storage conditions.

Seed weight (gm)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	282 a	282 a	282 a	282 a	282 a	282 a	282 a
	15	159 m	271 b	237 c	199 g	220 d	173 k	273 b
	30	159.3 m	212 e	183 j	191 h	212 e	172 k	200 g
	45	100 p	204 f	153 n	183 j	186 i	167 l	115 o

Table 8 : Total soluble solids of pomegranate fruit juice as affected by aloe vera gel and almond or coconut oil under storage conditions.

Total soluble solid (TSS, %)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	13.38 k	13.38 k	13.38 k	13.38 k	13.38 k	13.38 k	13.38 k
	15	16.20 j	16.50 fg	16.60 dg	15.85 j	15.90 ij	16.67 def	16.45 fgh
	30	16.98 cde	17.15 bc	17.15 bc	16.30 f-j	16.00 hij	16.35 f-i	16.53 efg
	45	17.15 bc	17.83 a	17.20 bc	17.95 a	17.03 cd	17.35 bc	17.50 ab

Table 9: Total acidity of pomegranate fruit juice as affected by aloe vera gel and almond or coconut oil under storage conditions.

Total acidity (TA, %)	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	1.33 a	1.33 a	1.33 a	1.33 a	1.33 a	1.33 a	1.33 a
	15	1.26 ab	0.83 a-e	1.15 abc	1.15 abc	1.28 a	1.22 a	0.90 a-e
	30	0.99 a-d	0.52 cde	0.88 a-e	0.90 a-e	0.70 a-e	0.92 a-e	0.043 de
	45	0.50 de	0.33 e	0.58 cde	0.63 b-e	0.35 e	0.47 de	0.138 de

Table 10 : Pectinase activity in fruit pulp of pomegranate as affected by aloe vera gel and almond or coconut oil under storage conditions.

Pectinase U/g pulp	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	1.602	1.602	1.602	1.602	1.602	1.602	1.602
	15	2.161	2.01	2.302	6.735	4.543	4.9	7.413
	30	4.021	3.442	3.743	7.413	6.735	5.855	9.864
	45	6.875	4.021	5.488	9.464	8.455	7.566	12.565

Table 11: Antioxidant activity of pomegranate fruit pulp as affected by aloe vera gel and almond or coconut oil under storage conditions.

Antioxidant Activity µg/ml juice	Storage days	Treatments						
		Aloe vera gel 10% + almond oil	Aloe vera gel 15% + almond oil	Aloe vera gel 20% + almond oil	Aloe vera gel 10% + coconut oil	Aloe vera gel 15% + coconut oil	Aloe vera gel 20% + coconut oil	control
	Zero	19.8 g	19.8 g	19.8 g	19.8 g	19.8 g	19.8 g	19.8 g
	15	31.6 ef	20.38 g	21.4 fg	44.2 c	34.21 e	31.1 e	24.1 f
	30	53.6 b	39.56 d	29.64 f	58.5 b	43.25 cd	39.67 d	33.15 e
	45	75.7 a	51.2 b	36.4 de	67.5 a	50.4 bc	54.7 b	40 d

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