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Effects of cold storage on quality parameters and nutraceutical compounds of pomegranate fruits (cv. Acco)

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Key words: Bioactive compounds, cold storage, *Punica granatum* L., quality fruit.

Abstract: *Punica granatum* L. contains several bioactive compounds with antioxidant activity that have a positive effect on human health. This study aims to investigate the changes in the chemical-physical and qualitative parameters of pomegranate fruits cv. Acco from harvest up to +90 days of cold storage (+4°C and 95% RH). Morphological parameters, juice yield, weight loss, total soluble solids content (TSS), pH, titratable acidity, the color of the epicarp (L*, a*, b*), content of polyphenols, anthocyanins, flavonoids, and antioxidant activity were analyzed. The results showed an increase (about 29%) in the juice content (%) at +60 days of cold storage. Cold storage has also shown positive effects on some bioactive compounds. Flavonoids and anthocyanins content increased from 287.98 mg CE/100 ml of juice to 389.23 mg of CE/100 ml of juice and from 8.32 to mg/100 ml of juice to 11.13 mg/100 ml of juice at + 90 days of cold storage, respectively. On the basis of our results that confirmed the literature data, the pomegranate fruit is rich in bioactive compounds that exert beneficial actions on human health, and it has also been demonstrated that such nutraceutical compounds increased during cold storage, allowing the fruit to be preserved a long term.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

The pomegranate (*Punica granatum* L.) is generally cultivated in the Mediterranean Basin, in the regions of Southern Asia, in India and in North and South America, where long and hot summer favor an optimal fruits ripening (Erkan and Dogan, 2018). Its adaptation to the Mediterranean climate has favored its spread in several countries giving rise, over the centuries, to numerous local genotypes. The fruits are generally harvested when fully ripe and displayed a smooth shining leathery skin with a color varies from green, to pink, reddish, or dark red (Love *et al.*, 2014). The pomegran-

ate fruits have a low respiration rate and a non-climacteric respiratory pattern (Ben-Arie *et al.,* 1984). The edible part of the fruit is called arils and constitutes about 52% of total fruit (w/w), comprising 78% juice and 22% seeds. The fresh juice contains 85.4% moisture and considerable amounts of total soluble solids (TSS), total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins (El-Nemr *et al.*, 1990) and has also been reported to be a rich source of antioxidants (Gil *et al.,* 2000; Kulkarni *et al.,* 2004). The pomegranate is highly valued for health-promoting benefits of its fruit and processed product as demonstrated by numerous *in vivo* and *in vitro* studies (de Nigris *et al.,* 2007; Bell and Hawthorne*,* 2008; Davidson *et al.,* 2009). The consumers pose more attention to the nutraceutical value of fruits, in fact several studies aim to valorize this aspect, as reported by Graziani *et al.* (2020, 2021). The recent growing awareness of consumers to health aspects of fresh fruits and processed products greatly increased the interest in consumption of this fruit and its processed products; consequently, worldwide pomegranate production expanded considerably. Apart from its demand as fresh fruit and juice, the processed products such as carbonated drinks, syrup, wine, and candy are also gaining importance in world trade (Dak and Pareek*,* 2014). The fruit juice of pomegranate was found to have an exceptionally high antioxidative capacity (Reddy *et al.,* 2007; Cirillo *et al.*, 2022). The shelf-life of pomegranate is about 12-14 days at ambient conditions (Naveena *et al.*, 2008); but cold storage with recommended temperature from 0 to 10°C can be used from 2 weeks to 5 months influencing in different manner cultivars storability (Ehteshami *et al.*, 2019). Storage method is very important because physiological and enzymatic processes cause the loss of quality with browning of the skin, necrotic pitting, pallor of the arils that depreciate the product during storage (Fawole and Opara, 2013 a; Dorostkar and Moradinezhad, 2022). In the present study, cv. Acco pomegranate storability, one of the main cultivars marketed in Italy, was analyzed, evaluating physical-chemical and biochemical changes during refrigerated storage up to 90 days from harvest to define the maximum storage time so that the fruits are still appreciated by consumers both organoleptic and nutritional point of view.

2. Materials and Methods

Experimental orchard and sampling

The experiment was conducted in 2019 in Eboli (Salerno, Italy) (40° 33’ 29” N; 14° 58’ 28” E a 15 m a.s.l.) at “Improsta” Regional Experimental Farm, where were cultivated the pomegranate trees of cv. Acco. The trees were trained to sapling system and spaced 3.5 m on the rows and 4 m between the rows. Pomegranate fruits were hand-harvested 3th September 2019 according to a randomized block design from homogeneous trees, for grown and production load. Fruits (n=120) were collected, and 12 lots (10 fruits each) were prepared. Three lots were analyzed at harvest while the others were stored in the cold chamber at a temperature of 4±1°C and 95±1% relative humidity, and analyzed after 30, 60 and 90 days of storage. In this work, 0 indicates the harvest time and +30, +60 and +90 the days of refrigerated storage.

Pomological and physico-chemical traits of fruits

Pomological and physico-chemical traits of the fruits were carried out at the Pomology Laboratory of the Department of Agriculture of the University of Naples “Federico II”. Fruit and seed weight (g) was determined by an electronic digital balance (Precisa Instruments AG, model XB220A, Dietikon, Switzerland) to monitor the weight loss of fruits during cold storage. Equatorial diameter (mm) and fruit length without calyx (mm) were measured by electric digital caliper with ±0.01 mm accuracy (Mitutoyo, Kawasaki, Japan). Color of epicarp was determined with a colorimeter (Minolta, model CR-400, Tokyo, Japan) that was capable of quantifying colors according to international standards and expressed in defined color spaces. The instrument was calibrated with “white” managed by the light source on a white tile, before each measurement. The L* a* b* (CIELAB) color space is the most common method for measuring the color of an object or materials of different origins and it is widely used in all sectors. In this color space, L* indicates brightness, while a* and b* the chromaticity coordinates: +a* is the direction of red, - a* is the direction of green, +b* is the direction of yellow, and - b* is the direction of blue. The measuring was repeated four times in different points of the fruit. The pomegranate seeds were hand-separated from the epicarp and carpellary membranes, counted, and squeezed using a small press. A juice yield of 300 g of pomegranate seeds was measured and expressed as a percentage (w:v). Juice (200 mL) was stored at -20°C and afterwards used for the evaluation of physico-chemical and nutraceutical traits. The pH, titratable acidity (TA) and total soluble solids (TSS) were assessed on the arils juice. The TSS con-

tent was determined with a HI 96.814 digital refractometer of Hanna instruments and results were expressed as °Brix. The pH was determined with a pH meter by the Hanna Instruments laboratory and total acidity was evaluate by acid-base titration, with 0.1N sodium hydroxide standard solution and the results were expressed as g citric acid 100 mL^{-1}.

Determination of total phenolic content

Total phenolics content in juice was determined according to a Folin-Ciocalteu procedure (Singleton and Rossi, 1965). The assay was carried out in duplicate for each sample using 5 µL of extract, 100 µL of Folin-Ciocalteu reagent and 300 µL of 7.5% (w/v) Na_2CO_3 solution; the mixture assay was left in the dark for 2 hours at room temperature, then the absorbance was determined at the wavelength of 765 nm The results were expressed as mg of gallic acid equivalent/100 mL of juice (mg of GAE/100 mL of juice).

Determination of flavonoids content

The assay was carried out according to Zhishen *et al.* (1999) by the aluminum chloride colorimetric method using 20 µL of juice and the absorbance was determined at 510 nm. Results were expressed as mg of catechin equivalent/100 mL of juice (mg CE/100 mL of juice).

Determination of anthocyanins content

The assay was carried out according to Magri *et al.* (2020) by a pH-differential method using 50 µL of juice in KCl pH 1 and CH_3COONa pH 4 buffer. The absorbances at 510 and 700 nm were determined. Results were expressed as mg of cyanidin-3-glucoside equivalent/100 mL of juice (mg C_3G/100 mL of juice).

Antioxidant activity

The assay was conducted as described by Petriccione *et al.* (2015) using 20 µL of juice and 1480 µL of 1,1-diphenyl-2-picril-hydrazyl (DPPH). The change in absorbance was observed at 515 nm and the results were expressed as mg of Trolox equivalent/100 mL of juice (mg Teq/100 mL of juice).

Statistical analysis

Analysis of variance (ANOVA) on the complete randomized block design on the data and mean separation by Duncan's multiple range test ($p<0.05$) and Principal component analysis (PCA) were carried out using XLSTAT, version 2013, statistical software package (New York, NY, USA).

3. Results

Fruit pomological characterization

The changes of the pomological parameters in the cv. Acco pomegranate fruits during the refrigerated storage at + 30, + 60 and + 90 days are shown in table 1. There is a significant reduction in the fruit weight loss about 10.5% already after 30 days of cold storage, up to 23.7% at +90 days, while no significant differences are highlighted for the equatorial diameter, the fruit length, and the seeds weight. One of the most important commercial parameters is the juice content in the seeds, our study showed a significant increase in the percentage of juice from collection to storage in the fridge, after +60 days about 63.5% compared to harvest (Table 1).

Juice quality parameters and fruit color characterization during cold storage

The results of the physico-chemical properties of cv. Acco during cold storage are shown in table 2. A significant reduction in total soluble solids (TSS) was highlighted, from 14.25°Brix at harvest to 11.85°Brix already at +60 days of refrigerated storage, up to a

Table 1 - Pomological parameters (fruit weight, equatorial diameter, fruit length, seed weight (n=100), % weight loss, % juice) at harvest and during the refrigerated storage (+30, +60, +90 days) of pomegranate fruits (cv. Acco). 0 days is the time of harvest

Days	Fruit weight (g)	Equatorial diameter (mm)	Fruit length (mm)	Seed weight (g)	Weight loss (%)	Juice (%)
0	216.38 ± 9.86 a	80.38 ± 1.53 a	63.5 ± 4.12 a	113.88 ± 5.76 b	0 ± 0.00 a	47.60 ± 0.47 c
30	187.18 ± 6.68 b	78.63 ± 0.92 a	65.5 ± 0.79 a	111.44 ± 4.86 b	10.5 ± 0.85 b	54.43 ± 1.55 b
60	171.27 ± 8.84 b	80.31 ± 1.35 a	67.0 ± 1.32 a	113.38 ± 6.70 b	19.6 ± 1.76 bc	63.50 ± 1.69 a
90	167.13 ± 7.48 b	80.88 ± 1.15 a	69.56 ± 0.73 a	119.87 ± 4.70 b	23.7 ± 2.10 c	61.35 ± 1.63 a
Significance	***	NS	NS	NS	**	***

All the data are expressed as mean ± SE (standard error). The same letter indicates not significant differences according to Duncan's multiple range test ($p<0.05$). Level of significance at the ANOVA are indicated as NS (not significant), * ($0.01<P<0.05$), ** ($0.01> P> 0.001$), and *** ($P<0.001$).

Table 2 - Total soluble solids (TSS), titratable acidity (TA), pH and color attributes (L*, a* and b*) at harvest and during cold storage (+30, +60 and +90 days) of pomegranate fruits (cv. Acco). 0 Days is the time of harvest

Days	TSS (Brix°)	TA (g citric acid 100 mL^{-1})	pH	L*	a*	b*
0	14.25 ± 0.25 a	8.75 ± 0.25 a	3.00 ± 0.00 b	47.08 ± 0.66 a	47.14 ± 0.88 a	26.98 ± 0.49 a
30	13.78 ± 0.09 a	8.10 ± 0.11 a	3.10 ± 0.09 b	40.51 ± 1.68 b	43.47 ± 1.32 b	20.66 ± 0.62 b
60	11.85 ± 0.72 b	6.60 ± 0.14 b	3.53 ± 0.07 a	39.09 ± 0.87 b	44.14 ± 0.55 b	20.63 ± 0.74 b
90	10.58 ± 0.21 c	5.63 ± 0.31 c	3.63 ± 0.07 a	41.21 ± 1.08 b	40.03 ± 0.56 c	20.68 ± 0.75 b
Significance	***	***	***	***	***	***

All the data are expressed as mean ± SE (standard error). The same letter indicates not significant differences according to Duncan's multiple range test (p<0.05). Level of significance at the ANOVA are indicated as NS (not significant), * (0.01<P <0.05), ** (0.01> P> 0.001), and *** (P <0.001).

final reduction of about 25.75% at +90 days while the pH showed an increase at +60 days of refrigerated storage equal to 17.66%. Color coordinates (L*, a*, b*) of pomegranate peel during the refrigerated storage are shown in Table 2. L* and fruit peel redness (*a**) showed a reduction with progressed refrigerated storage. The highest a * index was found at harvest (47.14), while after + 90 days of refrigerated storage this parameter was reduced to 40.03. b* was 26.98 at harvest while after + 30 days of refrigerated storage a reduction of about 23.42% was observed, without significant differences up to 90 days of storage.

Content of bioactive compounds and antioxidant activity during cold storage

The current use of pomegranate fruit regards especially the nutritional and, still potential, health benefits that come out from the various parts composing this one (carpellary membranes, arils, seeds and bark). Indeed, the phytochemical composition of the fruit abounds in compounds (flavonoids, ellagitannins, proanthocyanins, mineral salts, vitamins, lipids, organic acids) presenting a significant biological and nutraceutical value. Table 3 shows the bioactive compounds of the pomegranate fruits cv. Acco during the refrigerated storage. The polyphenol content did not display significant differences up to 60 days of storage, with average values of 272 mg GAE/100 ml of juice, while at 90 days an increase in the polyphenol content was shown up to a value of 389.23 mg GAE/100 mL juice; the flavonoid content showed a small increase at 30 days of storage (124.58 mg EC/100 mL juice), without statistical significance, for the other two periods considered with an average content of 103 mg CE/100 mL juice; the anthocyanin content displayed a slight decrease up to 60 days of refrigerated storage with average values of about 3 mg C_3G/100 mL of juice, followed by an increase after 90 days, up to 11.13 mg C3G/100 mL of juice. The antioxidant activity of pomegranate

Table 3 - Total polyphenol, anthocyanins, flavonoids content and antioxidant activity (D) at harvest and during cold storage (+30, +60 and +90 days) of pomegranate fruits (cv. Acco). 0 Days is the time of harvest

Days	Polyphenols (mg GAE/100 mL juice)	Anthocyanins (mg C_3G/100 mL juice)	Flavonoids (mg CE/100 mL juice)	Antioxidant activity (µmol TE/ 100 mL juice)
0	287.98 ± 8.86 b	8.32 ± 1.67 a	99.49 ± 3.25 a	271.63 ± 4.13 a
30	285.25 ± 26.07 b	3.53 ± 2.67 b	124.58 ± 9.27 a	268.11 ± 5.56 a
60	244.15 ± 23.92 b	2.72 ± 3.36 b	102.99 ± 1.90 a	267.67 ± 3.49 a
90	389.23 ± 29.01 a	11.13 ± 4.71 a	104.21 ± 10.46 a	267.97 ± 3.21 a
Significance	*	**	NS	NS

All the data are expressed as mean ± SE (standard error). The same letter indicates not significant differences according to Duncan's multiple range test (p<0.05). Level of significance at the ANOVA are indicated as NS (not significant), * (0.01<P <0.05), ** (0.01> P> 0.001), and *** (P <0.001).

juice showed no significant differences during the refrigerated storage with average values of 268 µmol TE/100 mL juice.

Principal component analysis (PCA)

To obtain a broad overview of all parameters evaluated in cv. Acco fruit following the refrigerated storage a principal component analyses (PCA) was conducted. Figure 1 shows the PCA of the changes of nutraceutical compounds and qualitative parameters, during refrigerated storage. The first two principal components (PCs) disclosed 88.39% of the cumulative variance with PC1 detailing for 55.55% and PC2 for 32.83%. At harvest this cultivar showed a higher antioxidant activity, while a higher flavonoids content is shown between +30 and +60 days of refrigerated storage, at +90 days of refrigerated storage a higher polyphenols and anthocyanins content was highlighted. The qualitative parameters showed higher values at +30 days of refrigerated storage for TSS and TA, while at + 60 days there was a higher juice yield.

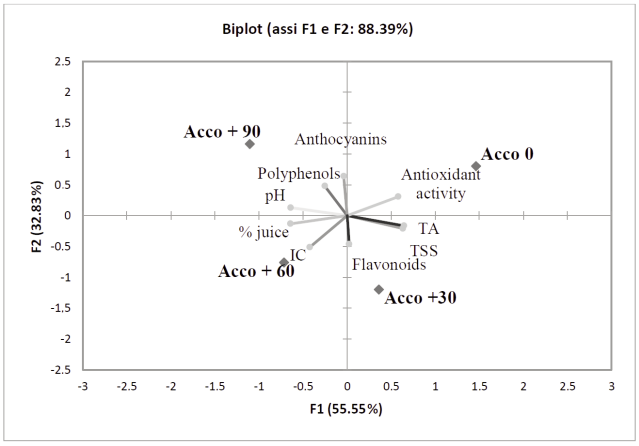

Fig. 1 - Principal component analysis (PCA) based on quality parameters and bioactive compounds at harvest and during cold storage (+30 +60 and +90 days) of pomegranate fruits (cv. Acco). 0 is the time of harvest.

4. Discussions and Conclusions

Both fresh market and processing industry drive pomegranate consumption, and it is crucial to acknowledge all fruit characteristics to not only classify varieties from a botanical point of view, but also to meet the current market demand for quality fruits (Martìnez *et al.*, 2006). In the recent years, the demand and the consumption of pomegranate fruit have shown an extensive growth in several countries worldwide becoming a valuable commodity due to high levels of nutraceutical compounds and greater awareness of health-promoting benefits (Asgary *et al.*, 2022). The major postharvest losses can occur among harvest and consumption affecting either fruit quantity or quality at any stage in the postharvest chain. Freshly harvested agricultural products are a living thing that breathes and undergoes changes during postharvest handling (Kiaya, 2014; Erkan and Dogan, 2018; Siddiqui *et al.*, 2022). Postharvest diseases caused by bacteria, fungi, and other microorganisms are compromised factors and a consistent problem for long term storage of pomegranate fruits (Munhuweyi *et al.*, 2016; Akhila *et al.*, 2022; Gurtler and Garner, 2022). These spoilages greatly affect the appearance, aroma and taste of fruits and the control of postharvest diseases represents the most significant economic challenges in agriculture. High water and sugars content combined with soft and delicate texture make pomegranate fruits susceptible to weight loss, mechanical damage, and attack of pathogens (Sayarri *et al.*, 2012). Physiological and enzymatic disorders are responsible to qualitative decay and storage life in pomegranate like fruit wrinkling, browning, and drying of skin and seeds, seeds paleness and the pathogens often that cause damage to the tissues, thereby making the fruit unsaleable (Caleb *et al.*, 2012 a; Nazoori *et al.*, 2022). As previously mentioned, an important aspect is the juice content. Our results reported an increase in the juice content during refrigerated storage most likely due to the "softening" of the tissues of the arils increasing the extraction of the juice. At harvest, pomological and physico-chemical traits in cv. Acco fruit showed a slight difference compared to what is reported by Ferrara *et al.* (2014); these differences may be ascribed to different cultural practice, climatic and soil conditions (Fadavi *et al.*, 2005; Ferrara *et al.*, 2014). Weight loss is one of the major problems associated with stored pomegranate fruit which cause hardening of the skin and browning of the rind and seeds (Artés *et al.*, 2000 a; Caleb *et al.*, 2012 b; Pareek *et al.*, 2015). Several studies have demonstrate that weight loss increased with increasing temperature and prolonged storage in different pomegranates cultivars and it is due to water being lost through natural porosity of the skin (Al-Mughrabi *et al.*, 1995; Al-Yahyai *et al.*, 2009; Wasker, 2011; Fawole and Opara, 2013 b). Our results, on the physico-chemical parameters of pomegranate fruits during cold storage, are in agree with those reported in the

literature where a reduction in TSS and TA is shown. According to a similar study on 'Mollar' pomegranate after only 7 days of storage at 4°C, the TSS content is reduced about 11% (Gil *et al.*, 1996), while Artes *et al.* (2000 b) showed a significant reduction in the acidity of pomegranate fruit juice in cv. Molla de Elche stored at 5°C for 90 days and subsequently, held at 20°C for six days. The decrease in TSS content could be a result of the degradation of sugars with prolong storage period and the changes in TA levels are strong indications of the ongoing metabolism in the fruit during storage since pomegranate is a non-climacteric fruit (Fawole and Opera, 2013 a). The decrease in fruit acidity can be attributed to the cumulative effects of the increase in juice content and the use of organic acids which act as a substrate for cellular respiration that occurs during fruit ripening (Diakou *et al.*, 2000). Organic acids present in pomegranate include citric, malic, acetic, fumaric, tartaric and lactic acid, but citric acid is the main one which represents the titratable acidity of pomegranate fruits (Melgarejo *et al.*, 2000). The results obtained on the peel color changes during refrigerated storage are in agreement with the results obtained by other studies, where similar storage conditions on 'Ganesh' pomegranate fruit induced the slight change in stored fruit color over a 12-week duration (Nanda *et al.*, 2001). Furthermore, during cold storage our findings highlighted a reduction of fruit lightness and a darker and saturated red color of the skin. In addition to being appreciated for its qualitative aspects the pomegranate is known for the high nutritional value of its fruits and for its beneficial effects on health, and the medicinal properties of the different parts of the tree are also well known. The main phytochemicals responsible for these beneficial health effects are polyphenols which include ellagic acid, ellagitannins (eg punicalagin), punicic acid, anthocyanins, flavonols, flavan-3-oils and flavones. Ellagic acid can be free or condensed with different sugars (glucose, rhamnose and arabinose), with different concentrations between various cultivars (Zaouay *et al.,* 2012). Although ellagitannins are the main polyphenols in pomegranates, punicalagin and punicalin are the compounds most characterized for their antiatherogenic properties (Seeram *et al.*, 2005). Anthocyanins are the pigments responsible for the typical pomegranate fruit color and include delphinidin, cyanidin and pelargonidin 3-glucoside and 3,5-diglucosides. The changes in the composition and concentration of anthocyanins have been shown in the different cultivars, the main anthocyanin in the Spanish sweet pomegranate cultivar "Mollar de Elche" is cyanidin 3-glucoside, while cyanidin 3,5-diglucoside has been found as the main compound in sour pomegranate cultivars (Sayyari *et al.*, 2011 a, b). Several studies highlighted the changes in the bioactive compounds content during the refrigerated storage of the pomegranate (Zaouay *et al.,* 2012; Ehteshami *et al.*, 2020). Fawole and Opara (2013 a) have shown that the polyphenol content did not vary during the first 4 weeks of storage in the 'Bagwa' fruit but increased after 8 weeks due to an accumulation of anthocyanins; variations of bioactive compounds during cold storage are also shown in apples fruits (Graziani *et al.,* 2020). In our study, no decrease in polyphenol content was observed during refrigerated storage in agreement to Baltacioglu *et al.* (2011) and this is attributed to the action of oxidative enzymatic activities following low temperature stress (Ehteshami *et al.*, 2020). The anthocyanin content in the pomegranate showed different trends related to the cultivars and storage temperatures (Fawole and Opara, 2013 a), which suggests that varietal differences represent a key factor in the post-harvest biosynthesis of anthocyanins (Turfan *et al.*, 2011). Furthermore, several studies have shown that the antioxidant activity is linked to the bioactive compounds content and that a decrease in this activity is observed in the pomegranate during refrigerated storage after 4 weeks compared to harvest (Fawole and Opara, 2013 a). Our study showed during refrigerated storage a significant weight loss, while the juice percentage increased, with maximum values at +60 days. Acidity (TA) and TSS decreased during refrigerated storage, but the reduction in acidity was more significant than that of TSS, therefore overall good organoleptic characteristics were showed. The polyphenols and anthocyanins increased during refrigerated storage, while the flavonoids and antioxidant activity were constant. On basis of our results suggest that pomegranate fruits cv. Acco have a maximum storage time in the fridge of 60 days for fresh consumption while they can exceed 60 days to produce the juice, as the chemical-nutraceutical characteristics remain optimal. During the cold storage the pomegranate fruits of the cv. Acco cultivar did not show the appearance of plant diseases (molds or rot fungi), thus allowing a good conservation of the product.

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