Assessment of light conditions effect on nutritional quality and bioactive compounds of Moroccan clementine juice during cold storage

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Abstract
Citrus juices hold the leading position among juices in Moroccan global consumption. The nutritional quality of juice is mainly related to its polyphenols and vitamin C contents, and antioxidant properties. The degradation of these bioactive compounds might be a critical factor for juice quality. The objective of this investigation is to assess the effect of light conditions (daylight, lamp light and darkness) on the physicochemical and nutritional quality of clementine juice during 8 storage periods starting from 1 day until 3 months at 4°C. Thus, physicochemical parameters (pH, Brix, and color), bioactive compounds (total polyphenols and vitamin C) and total antioxidant activity (AA) were evaluated during storage. Results showed that after 7 days of storage under light, clementine juice become chemically instable and the nutritional quality loss is highly significant. After 3 months of storage under daylight and lamp light, loss of vitamin C and polyphenols contents was observed exceeding 80 % and 60 %, respectively, compared to fresh juice. However, less significant reduction was observed for juices stored in darkness (loss of 50 % for vitamin C and only 28 % for polyphenols). These losses affected highly the AA of the stored juice. In fact, total AA of freshly prepared juice was 54 %. However, after 3 months of storage under daylight and lamp light, the AA decreased to 15 % and 17 %, respectively. The decrease was less accelerated in dark condition (38 % of AA). Therefore, storage time and light are determining factors in the preservation of clementine juice quality and dark packaging is highly recommended.

Keywords: clementine juice, physicochemical criteria, Polyphenols, vitamine C, antioxidant activity, storage light, Morocco.
Évaluation de l'effet des conditions de lumière sur la qualité nutritionnelle et les composés bioactifs du jus de clémentine Marocain pendant le stockage au froid

Résumé
La qualité nutritionnelle des jus des agrumes est liée essentiellement à sa teneur en polyphénols et en vitamine C, et à ses propriétés antioxydantes. La non-maîtrise des conditions de conservation et de stockage pourrait être un facteur critique provoquant la dégradation de ces composés bioactifs et induisant la dépréciation de sa qualité nutritionnelle. Ainsi, l'objectif de cette étude est d'évaluer l'effet des conditions de lumière durant le stockage sur la qualité physicochimique et biochimique du jus de clémentine. Le jus extrait est stocké sous trois conditions : lumière du jour, lumière de la lampe et obscurité pendant 8 périodes allant d'un jour à 3 mois à une température de 4 °C. Les paramètres physicochimiques (pH, Brix et couleur) et les composés bioactifs (polyphénols totaux et vitamine C) ainsi que l'activité antioxydante (AA) ont été évalués au cours du stockage. Les résultats ont montré qu'après 7 jours de stockage sous la lumière, le jus de clémentine devient chimiquement instable et la perte de la qualité nutritionnelle est très significative. Après 3 mois de stockage à la lumière du jour et à la lumière de la lampe, une perte de la teneur en vitamine C et en polyphénols a été observée, dépassant respectivement 80 % et 60 % par rapport au jus frais. Cependant, une réduction moins significative a été observée pour les jus stockés dans l'obscurité (50 % pour la vitamine C et seulement 28 % pour les polyphénols). Ces pertes ont fortement affecté l'AA du jus stocké. En fait, l'AA du jus fraîchement préparé était de 54 % ; cependant, après 3 mois de stockage à la lumière du jour et à la lumière de la lampe, l'AA a chuté à 15 % et 17 %, respectivement. Cette diminution était moins accélérée dans l'obscurité (38 % de l'AA). Par conséquent, la durée du stockage et la lumière sont des facteurs déterminants dans la préservation de la qualité du jus de clémentine et le conditionnement à l'obscurité est fortement recommandé.

Mots-clés : jus de clémentine, critères physico-chimiques, polyphénols, vitamine C, activité antioxydante, stockage sous lumière, Maroc.
تقييم تأثير الضوء على الجودة الغذائية والمركبات النشطة بيولوجيا لعصير الكليمنتين المغربي أثناء التخزين البارد

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ملخص

ترتبط الجودة الغذائية لعصائر الفواكه الحمضية أساسًا بحتواها من مادة البوليفينول وفيتامين ج وخصائصها المضادة للأكسدة. يمكن أن يكون عدم احترام شروط الحفظ والتخزين عاملاً حاسماً يتسبب في تدثر محتوى هذه المركبات البيولوجية ويؤدي إلى انخفاض جودتها الغذائية. إن الهدف من هذه الدراسة هو تقييم تأثير ظروف التخزين تحت الضوء على الجودة الفيزيوكيميائية والبيوكيميائية لعصير الكليمنتين. تم تخزين العصير في ثلاثة ظروف (ضوء النهار، ضوء المصباح والظلام) لمدة تتراوح بين يوم واحد وثلاثة أشهر تحت 4 دراجات مئوية. تم تقييم التغيرات الفيزيوكيميائية (الرقم الهيدروجيني والبركس واللون) والمركبات البيولوجية (البوليفينول الكلي وفيتامين C) إضافة إلى النشاط المضاد للأكسدة (AA) خلال فترة التخزين. أظهرت النتائج أن بعد 7 أيام من التخزين في الضوء، يصبح عصير الكليمنتين غير مستقر كيميائياً ويصبح فقدان الجودة الغذائية كبيرًا للغاية. بعد 3 أشهر من التخزين في ضوء النهار وضوء المصباح، ورحت نقص في فيتامين C وحوالي البوليفينول بما يتجاوز 80% و60% على التوالي مقارنة بالعصير الطازج. إلا أنه لوحظ انخفاض أقل أهمية للعصير المخزن في الظلام (50%). كانت نسبة AA لفيتامين ج و28% فقط للبوليفينول). أثرت هذه الانخفاضات بشدة على AA للعصير المخزن 54٪، بينما بعد 3 أشهر من التخزين في ضوء النهار وضوء المصباح، انخفض AA إلى 15٪ و17٪ على التوالي. كان هذا الانخفاض أقل تسارعاً في الظلام حيث سجل AA 38٪. لذلك، يعد مدة التخزين والضوء عاملان حاسمان في الحفاظ على جودة عصير الكليمنتين كما يوصى بشدة عدم الضوء أثناء التخزين.

الكلمات المفتاحية: عصير كليمنتين، معايير فزيائية كيميائية، بوليفينول، فيتامين ج، نشاط مضاد للأكسدة، ضوء التخزين، المغرب.
Introduction

The citrus fruit sector plays an important socio-economic role in most producing countries around the world. In Morocco, the citrus fruit sector has undergone significant development over the last two decades thanks to the efforts made through the strategy of Green Morocco Plan to modernize agriculture and increase agricultural production. Indeed, the citrus occupies an area of about 128 000 hectares with an annual average of about 2.4 million tons, and exports estimated at an average of 644 000 T, which generate a revenue of more than 3 billion DH / year (MAMPDREF, 2019). The varietal profile of the Moroccan citrus orchard is composed of a diverse range of varieties, but is still dominated by 3 groups of varieties, namely, the clementine group with 35 %, Maroc Late with 21 % and Navels with 18 %.

Despite its development, the sector has experienced major problems in recent years. According to the Moroccan Ministry of Agriculture annual report of 2019, citrus fruit production for the 2018/2019 season reached approximately 2.6 million tons, an increase of 18 % over the previous marketing year. The largest increase recorded in the areas of Berkane and Beni Mellal, where production increased by 25 % and 18 %, respectively. In the face of this significant increase in production, the instability of export markets and the insufficient number of citrus processing units in Morocco, about 60 % of national production remains in the local market, mainly clementine varieties, causing a flooding of the market and a collapse in selling prices, leading to deplorable socio-economic problems.

In this context, Morocco has invested in a new strategy "Green Generation 2020-2030", one of the major objectives of which is to continue the dynamic development of agricultural sectors, by strengthening production chains and developing the downstream agricultural value chain through processing to cover 70 % of production. Thus, INRA has programmed research projects that aim to set a clementine juice process and to evaluate its stability through storage conditions. The present study is part of these research activities.

Among fruits and fruit products, oranges and orange juices are known as an important source of vitamin C and polyphenolic compounds. Vitamin C is considered as a most important water-soluble antioxidant. It protects compounds in extracellular and intracellular spaces in most biological systems and reduces tocopherol radicals back to their active form at the cellular membranes (Kaur et al., 2001). Currently, ascorbic acid is the most widely used vitamin supplement worldwide. The vitamin C content in orange juices ranges from 150 to 450 mg/L (Gliszczynska et al., 2014). Moreover, an important quality loss of orange juice during shelf-life is the change in color. It is the first visible sign, which can negatively influence consumer acceptance, thereby decreasing its commercial value (Manso et al., 2018). It is known that color change is an indicator for chemical and biochemical reactions (Van Boekel et al., 2008). Depending on the extent of the reaction, changes in color may occur because of the formation of brown pigments as well as the fading of carotenoids, the naturally occurring pigments of orange juice. Therefore, there is important need to evaluate and assess clementine juice physicochemical and biochemical stability under storage conditions to improve its shelf-life.
To the best of our knowledge, no previous works were reported on the effect of light storage conditions on the chemical stability of Moroccan clementine juice. Based on the above considerations, the main objective of the current study was to evaluate, assess and monitor the physicochemical and biochemical stability of clementine juice under light storage conditions (Daylight, lamp light and darkness) as function of time of storage during 3 months.

**Materials and methods**

**Vegetal material**

The variety of clementine used in this study is Sidi Aissa chosen for its quality and its very high production in the region of Beni Mellal - Maroc. Citrus fruit samples were collected during the period of November to December from four collection points in the Beni Mellal region (Domaine Agricole production stations and CRRAT production platform). Fruits were harvested from 20 trees in each collection point (from different parts of the tree (2kg/tree).

**Clementine juice processing**

The Juice processing protocol was based on general industrial preparations of Valencia juice with modification at laboratory scale (Azzouzi et al. (2018). Improvements and adjustments have been made through product stability studies to adapt the juice processing to clementine fruit. The process of clementine juice production is summarized in figure 1.

![Figure 1: Preparation of clementine juice at laboratory scale](image-url)
The clementine fruits were washed by immersing in water during 2 min; then, cleaned under water, drained and air-dried. The fruits were treated at a temperature of 77 °C for 5 min (bleaching). The extraction of juice was performed using “citrus juicer” (juice extractor by press intended to squeeze citrus juice). The clarification of the juice was performed by centrifugation of juice in a 2000 rpm/5 min. The obtained juice was collected in sterile bottles, and pasteurized at 88 °C for 3 min. The bottles were cooled and stored at 4 °C until analyses.

**Experimental approach**

In order to study the chemical stability of clementine juice stored under light conditions, the experimental approach presented in figure 2 was adopted.

![Experimental approach](image)

**Figure 2:** Global experimental approach adopted for juice quality and stability evaluation.

**Physicochemical analyses**

The pH was measured using a moving pH meter for food (Thermo Orien 3 Star, USA). The total soluble solids (TSS or Brix) was determined using a digital refractometer (Metteler-Toledo GmbH, Switzerland) at 20 °C. Results were expressed in °Bx (Kimball, 1991).

For color measurements (based on the CIELAB scale), A tristimulus colorimeter Chroma Meter CR-410 (Konika Minolta Sensing Inc., Osaka, Japan) was used to measure colorimetric parameters L*, a* and b* of clementine juices.

The L* axis (luminance) expresses the brightness, ranging from total black to total white. The a* and b* axes are the two-color coordinates, with the a* axis ranging from green to red and the b* axis ranging from yellow to blue.

Color measurement was performed on 30 mL samples in Petri dishes against a background of white tiles (Yawadio and Morita, 2007).
Bioactive compound analyses

**Determination of Vitamin C**

Vitamin C was determined using 2,6-Dichlorophenolindophenol (DCPIP) titration method (AOAC, 1995). The indophenol solution was standardized by titration with 2.0 mL of standard ascorbic acid solution and 5 mL of HPO3 + HOAc solution to the end point (a persistent rosypink color). The consumption of the blank was determined by titration indophenol solution with 7 mL of HPO3 + HOAc solution plus a given amount of water equivalent to the volume indophenol solution used in the previous standardization titration. For sample titration, a 100 mL portion of the juice was mixed with an equal volume of HPO3 + HOAc solution before filtering. A volume of the filtrate equivalent to about 250 mg of ascorbic acid was then titrated with indophenols solution using the same procedure as described above including the titration of the blank.

**Quantification of total polyphenols**

Total phenolic content in samples were determined as described by Singleton et al. (1965), with slight modification: 0.2 mL of extract juice was mixed with 1.5 mL of the Folin-Ciocalteu reagent diluted 1/10 and 1.2 mL of 7.5 % of the sodium carbonate. The mixture is incubated for 90 min in dark at room temperature after the absorbance is measured at 725 nm using a UV-visible spectrophotometer. Gallic acid was used as a calibration standard, and the results are expressed in mg EAG (Equivalent Gallic Acid).

**Antioxidant activity**

Antioxidant activity of clementine juice samples was determined following DPPH (2,2-diphenyl1-picrylhydrazyl) assay as described by Sanchez-Moreno et al. (1998) with a slight modification.

Briefly, 0.1 mL of juice sample (diluted with distilled water and centrifuged) was added to 2.46 mL of (DPPH; 0.025 g L in 50 % ethanol) and mixed by vortex for 5 min. The absorbance of the samples was measured at 515 nm every 1 min for 5 min using the spectrophotometer UV-Visible. For each sample, three separate determinations were carried out. The antioxidant activity was expressed as the percentage of decline of the absorbance after 1 min, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH, which was scavenged (%DPPHsc) was calculated using:

\[
\%DPPH_{sc} = \frac{(A_{cont} - A_{samp}) \times 100}{A_{cont}},
\]

Where \(A_{cont}\) is the absorbance of the control, and \(A_{samp}\) the absorbance of the sample. All the aforementioned analyses were carried out in triplicate.

**Statistical analysis**

All measurements were conducted in completely randomized design in triplicates (n=3), unless elsewhere specified. Multiple comparison tests (Minimal Significant Difference test (LSD)) were performed by SPSS software version 2.0 to determine significant differences between group averages. The probability \(P < 0.05\) was considered statistically significant.
Results and discussion

Physicochemical parameters of Clementine juice

The physicochemical criteria (pH, Total soluble solids content and color parameters) of clementine juice stored under daylight, lamp light and darkness conditions during 3 months are presented in figures 3, 4, and table 1.

\textit{pH}

According to the results reported in figure 3, the pH value of fresh clementine juice was 3.42± 0.2. After 7 days of storage under lamp light and daylight condition, the pH value of clementine juice increased significantly ranging from 3.42 to 4.20 and 4.70, respectively. However, the samples stored in darkness were slightly modified (3.65). After 3 months of storage in (darkness; lamp light; Daylight) conditions, an increase in pH was observed from 3.42 to 4.5, 5.8 and 6.02, respectively. The obtained results showed that storage of clementine juice under lamp light and daylight for 3 months increased the juice pH value from 3.42 to 5.8 and 6, respectively. The change was less significant on samples stored in the dark. This change in pH under light will make the juice chemically unstable and may affect the microbiological stability of the product.

In fact, pH has a large impact on food preservation. Thus, the food alterations result from different types of chemical, enzymatic or microbiological reactions which themselves are influenced by the pH. The risk of bacterial growth is present in a range of pH = 4.5 to 9.0 while passing through an optimum of 6.5 to 7.5. Since pH of the juice became about 6, the risk of alteration due to the growth of bacteria is significant. The variation of pH value is related to the instability of acidity of the jus. This occurrence might be due to oxidation of acids during storage (Van Boekel \textit{et al.}, 2008).

\begin{center}
\includegraphics[width=\textwidth]{figure3.png}
\end{center}

\textbf{Figure 3}: Effect of storage conditions under daylight, lamp light and darkness on pH of clementine juice as function of time during 3 months of storage
**Total soluble solids content (TSS content / Brix)**

![Graph showing the effect of storage conditions on total soluble solids content](image)

**Figure 4:** Effect of storage conditions under daylight, lamp light and darkness on total soluble solids content (Brix) of clementine juice as function of time during 3 months of storage

According to results presented in figure 4, the Brix value of fresh processed clementine was 12 Bx. These results fall within the average sugar content of orange juice reported by previous investigators (Farnworth *et al.*, 2001; Kelebek *et al.*, 2009). After 7 days of storage, results showed an increase in brix samples stored in daylight and lamp light conditions (13.02 % and 13.10 %, respectively), however, the change of brix of samples stored under dark conditions remained more stable until 1 month of storage (13 %). After 3 months of storage in darkness, lamp light, and Daylight conditions, an important increase in Brix values was observed from 12 to 13.7, 15.0 and 15.2, respectively.

At the beginning of storage, the TSS content was 12 °Bx and at the end of storage, it increased by 3 °Bx under light condition (lamp light; day light) and with 1 °Bx under dark condition. Also, Kelebek *et al.* (2009) found a similar trend during refrigerated storage of orange juice for eight months under darkness. However, the authors did not mention possible reasons for this evolution. On the other hand, some studies found an insignificant change in the TSS content during storage of orange juice (Cortés *et al.*, 2008) and lemon juice (Robertson *et al.*, 1986) under darkness condition.

According to Wibowo *et al.* (2015), who reported a significant increase of Brix value of orange juice during storage at 20 °C and 42 °C for 32 weeks under darkness condition could be explained by the formation of soluble degradation products as a function of time and temperature.

**Color parameters**

In Figure 5, color parameters on the CIELAB scale (L*, a*, b*) were presented as a function of the storage conditions and time. According to results, the color of clementine juice was remarkably affected by storage under light and day light condition compared to darkness condition.
Figure 5: Color parameters (L*, a*, b*) changes of clementine juice as a function of storage conditions (daylight, lamp light, darkness) and time.
In fact, the initial L*, a*, b* values of fresh processed juice were 34.72, 14.69 and 5.37, respectively (figure 5).

After 1 week of storage under light conditions, remarkable increases were observed in a* and b* values of juice samples (15.59; 6.25) for lamp light and (15.89; 6.34) for daylight, however, a slight variation was observed until Two months of storage under dark condition.

After 3 months of storage, results showed a significant increase among all conditions (darkness; lamp light; daylight) with a* and b* values of (16.90, 7.01); (19.89, 10.30); (22.52, 11.90), respectively. The increased were more pronounced under light conditions, mainly daylight condition, compared to darkness.

Regarding L* variable, a remarkable decrease was revealed after 1 week of juice storage under light conditions, however, L* values of samples stored in darkness condition were generally stable. After 3 months of storage, L* values decreased significantly reaching values of (23.50) for lamp light and (21.05) for daylight.

Visual analysis of juices stored under daylight and lamp light showed a remarkable browning from two weeks of storage compared to samples stored in darkness, which showed a visible browning only from two months of storage. This confirms the results of the color parameter measurements (L*; a*; b*).

Our results are similar to those of Martí et al. (2002) who reported that the storage of unprocessed pomegranate juice at 25 °C caused a decrease in L* value resulting the darkest colors during the storage period.

Several studies have highlighted the relationship between pH values modifications of orange juice and the browning phenomena; in fact, acids may determine and influence some degradation reactions during prolonged storage. Kennedy et al. (1990) studied the influence of pH in the development of brown color of orange juice during storage. After 4 days of storage at 38 °C, a slight brown color was reported at pH 1.15–4.15 and yellow color at pH 7. Also, the effect of pH in the formation of 5-hydroxymethylfurfural (HMF), known as indicator for browning in citrus juices, was referred by Shallenberger and Mattick (1983) in which almost a 2-fold faster rate of HMF formation was found at pH 3 than at pH 4–6. Particular organic acids have been described to be of interest in literature, as it was suggested to contribute to the browning of orange juice (Shinoda et al., 2005). Thus, we can argue that the change in color parameters of the juice during storage in our study is enhanced by the change in pH during storage.

**Biochemical compounds of clementine juice**

The results of monitoring total polyphenols and vitamin C in orange juice samples stored under daylight, lamp light and darkness conditions for 3 months are shown in figures 6 and 7.
Total polyphenols content

According to results in figure 6, the total phenolics content of the fresh processed juice was 28.56 mg EGA/100mL. After 7 days of storage, a very significant decrease was noticed for all samples stored under days light and lamp light conditions (24.4 mg EGA/100mL; 24.7 mg EGA/100mL, respectively). However, the total phenolics content of clementine juice stored under darkness remained stable until 30 days of storage (24.94 mg EGA/100mL).

After 3 months of storage, we witnessed a loss of total polyphenols that exceeds 60% of the initial values of the samples stored under daylight and lamp light (8.89 mg EGA/100mL; 10.7 mg EGA/100mL). This loss was only 28% of the initial value in total polyphenols of the samples in the dark (20.11 mg EGA/100mL).

![Figure 6: Effect of storage conditions under daylight, lamp light and darkness on total phenolics content of clementine juice as function of time during 3 months of storage](image)

Kannan and Thirumaran (2001) also reported similar results for jamun products and Sharma et al. (2012) for guava-jamun blended RTS drink and squash. According to Karpagavalli et al., (2015), Phenolic compounds are highly volatile and easily oxidized. A gradual loss of total phenols during storage might be due to their condensation into brown pigments. This oxidation could explain the general losses of phenolics content and the browning phenomena observed during storage conditions and especially under light conditions.

Vitamin C content

According to results in figure 7, the vitamin C content of the fresh processed juice was 354 mg/mL. After 3 days of storage, a significant decrease was noticed for samples stored in lamp light and daylight (294 mg/mL, 291 mg/mL, respectively), however, vitamin C of clementine juice stored under darkness remained generally stable until 15 days of storage (301 mg/mL). After 3 months of storage under daylight, lamp light and darkness, Vitamin C decreased to 89 mg/L; 74 mg/L and 149 mg/mL, respectively. In other words, the rate of vitamin C losses of clementine juice reached values exceeding...
80 % in storage under daylight and lamp light, however, this rate reached about 50 % under darkness condition.

The results presented are in line with the data obtained by Kabasakalis et al. (2000) and Arena et al. (2001) who reported a significant decrease in ascorbic acid content in reconstituted juice from concentrate blood-orange juice stored in darkness at 25 °C during 60 days by about 25 %.

Vitamin C is considered the most important elements of the nutritional quality of orange juice. According to the literature data, the content of vitamin C in different juices decreases during storage, depending on storage conditions, such as temperature, oxygen and light access (Kabasakalis et al., 2000; Zerdin et al., 2003).

Figure 7: Effect of storage conditions under daylight, lamp light and darkness on vitamin C content of clementine juice as function of time during 3 months of storage

Antioxidant activity of Clementine juice

The results antioxidant activity in orange juice samples stored under daylight, lamp light and darkness conditions during 3 months are shown in Figure 8.
Figure 8: Effect of storage conditions under daylight, lamp light and darkness on antioxidant activity of clementine juice as function of time during 3 months of storage

According to the DPPH assay, the scavenging activity of fresh juice was 54%. These results are in agreement with those reported in a previous study (Malecka et al., 2003), in which orange juices scavenged 33–49% of DPPH radical. After a storage period of 7 days, results showed a significant reduction in antioxidant activity for all samples under day light and lamp light conditions (35%: 38%), however, the antioxidant of clementine juice stored under darkness remained generally stable until 30 days of storage (44%). After 3 months of storage, the decrease of antioxidant activity of clementine juice stored under light conditions (daylight; lamp light) reached 70% of reduction, however, the decrease was less significant in dark condition (only 29% of reduction). By analyzing the results, we can clearly notice that the decrease in polyphenol and vitamin C compounds upon storage under light conditions (daylight and lamp light) affected the decrease in DPPH antioxidant capacities of clementine juices.

The results presented are in line with the data obtained by Gardner et al. (2000) who observed that both vitamin C and total polyphenols in fruit juices strongly correlate with the antioxidant capacity determined in FRAP assay. In addition, many studies (Klimczak et al., 2007; Muzaffar et al., 2017) reported that the decrease in antioxidant activity could be related to decrease in vitamin C, total polyphenols and total flavonoid content degradation. The decrease in the antioxidant activity may be linked to a lower content of phenolic compounds and vitamin C in stored juice as compared to fresh.
Conclusion

Results indicated that, clementine juices stored under light conditions (daylight and lamplight) become chemically instable and losses in terms of nutritional quality are highly significant compared to darkness conditions.

The physicochemical parameters modification of the juice including pH, total sugar content and color parameters may be important factors leading to the degradation of polyphenols and vitamin C and decreasing the antioxidant activity of clementine juice. Thus, it can be concluded that clementine juice storage under light makes it chemically unstable and trigger several reactions that deteriorate the technological and nutritional quality of the juice.

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