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Health Potential and Physicochemical Attributes after Minimal Processing and during Refrigerated Storage of Orange (*Citrus sinensis* **L., Osbeck)**

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Changes after minimal processing and during refrigerated storage for10 days at 3◦*C of sweet oranges (cv. Valencia Late) were evaluated. Physicochemical and sensory attributes and health potential (ascorbic acid, total phenolics, and antioxidant capacity) were quantified. The minimal processing (in wedges and slices) did not cause significant changes in quality attributes. Ascorbic acid was retained by over 70% until day 10 of storage at 3*◦*C, total phenolics remained constant, and antioxidant capacity showed a significant decrease until day 5, and then remained constant. Sensory analysis indicated absence of surface dryness at day 10 and a moderate development of off-odors at day 7.*

KEYWORDS minimal processing, refrigerated storage, total phenolics, antioxidant activity, orange

INTRODUCTION

Argentina is one of the top ten producers of citrus, according to statistics published by the USDA (2010). Citrus cultivation is centered mainly in two regions: the central east, with a predominance of orange and tangerine, and the northwest with lemon and grapefruit.

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Citrus fruits are non-climacteric fruits, with relatively low respiration and ethylene production rates, not undergoing any major softening or compositional changes after harvest and, therefore, can normally be stored for relatively long periods of 6–8 weeks (Artés-Hernández et al., 2007).

The orange is a fruit with important nutrients and bioactive antioxidants compounds, including Vitamin C or ascorbic acid and its derivatives (Del Caro et al., 2004). The flavanone glycosides, such as hesperidin, naringin, and narirutin, are the most important water-soluble phenols. Other compounds, such as limonoids (triterpene derivatives); some flavones, such as sinensetin and nobiletin; and phenylpropanoids, such as hidroxycinnamates, have high antioxidant potential and health-promoting capacity (Kaur and Kapoor, 2001). According to Miller and Rice-Evans (1997), soluble phenolic compounds of orange juice are narirutin and hesperidin, but the most significant antioxidant is ascorbic acid.

The consumption of antioxidant compounds has recently been associated with a decreased risk of developing degenerative diseases, such as cancer, diabetes, and cardiovascular and neurological diseases (Del Caro et al., 2004). These features give the orange a high nutritional value and important beneficial health properties.

As a consequence of their convenience, freshness, and health-related attributes, many companies have been trying to promote the consumption of ready-to-eat products derived from citrus (Rocha et al., 1995; Del Caro et al., 2004; Rapisarda et al., 2006; Artés-Hernandez et al., 2007). The marketing of minimally processed citrus, obtained after simple operations on the whole fruit, could increase their consumption and be very attractive for use in both home and institutional settings (schools, hotels, restaurants, and others). However, the production of fresh-cut citrus leads to a decrease in shelf life. The main problem is that when cells are ruptured by cutting during minimal processing, the wound-induced biochemical reactions shorten the storage life (Artés- Hernández et al., 2007). When citrus fruits are peeled and the outer protective layer removed, the fresh cells rich in water, sugars, and organic acids are exposed. The leakage of nutrients will promote microbial growth and the damaged tissue will also provide a portal of entry for establishment of microbes. One might expect an increase in respiration rate in fresh-cut fruits and a shorter shelf life, as it varies inversely with the respiration rate (Rocha et al., 1995).

Moreover, Cisneros-Zevallos (2003) proposed that controlled abiotic stress treatments, such as wounding, temperature, altered gas composition, and heat shock, among others, may be used as tools by the fresh produce industry to enhance the health benefit properties of fresh-cut fruits and vegetables. Del Caro et al. (2004) found that minimal processing caused changes in the contents of flavonones, glycosides, and ascorbic acid and in the antioxidant capacity of citrus segments and juices. These changes differed on the basis of the kind of preparation. Plaza et al. (2011) found that although some vitamin C losses were observed, other bioactive compounds (carotenoids and flavanones) were retained in minimally processed oranges during refrigerated storage; Plaza et al. (2011) also concluded that further research is needed to provide more insight into the impact of minimal processing on the orange bioactive compounds.

Therefore, there is a scientific and technological challenge to guarantee the consumer that minimally processed fruits provide, at least, the same nutrients and compounds with biological activity that is found in the whole fruit. The aim of this study was to evaluate the quality changes after minimal processing and during the subsequent refrigerated storage of a commercial '*Valencia Late'* sweet orange, quantifying physicochemical attributes and their health potential.

MATERIALS AND METHODS

Plant Material

'Valencia Late' sweet oranges (*Citrus sinensis* L., Osbeck) from a commercial late harvest season from Chajarí in the northeast of Entre Rios province (Argentina) were used. The oranges were characterized by determining mean weight per fruit, percentage of peel, percentage of minimal processing yielding (peeled fruit weight*/*whole fruit weight), number of seeds, percentage of juice, ascorbic acid content, soluble solids content, total acidity, pH, brix*/*acid ratio, total phenols content, and antioxidant capacity. The whole oranges were cut in half, squeezed using a manual citrus juicer and filtered. This juice was used to calculate the percentage of juice and to perform the other assays to characterize the raw material.

Minimal Processing of Plant Material and Sample Preparation

Minimal processing included selection and elimination of oranges with signs of rot. Fruits with a weight greater than 150 g were used for processing. They were washed with tap water for 1 min, and then were immersed in NaClO 200 ppm aqueous solution, $pH = 7$ for 2 min. The fruits then received a heat treatment by immersion in water at 50◦C for 5 min. The fruits were peeled and cut into slices and wedges, and were then packaged into round rigid plastic containers with lids (volume: 270 cm^3 , PET 0.42-mm thick, transmission rates: $29-59$ [cm³/m².día.atm] for O₂ [at 23[°]C and 0% RH] and 12–18 [g*/*m2.día] for water vapor [at 38◦C and 90% RH]). Storage was at 3[°]C for 10 days. Samples were evaluated immediately after processing (day 0) and during refrigerated storage (3, 5, 7, and 10 days at 3◦C) in terms of: ascorbic acid content, total phenols content, antioxidant capacity, total acidity, soluble solids content, pH, brix*/*acid ratio, percentage of exudates (% E): liquid weight on the container bottom in relation to the initial weight of minimally processed fruit, percentage of weight loss (% WL): initial weight (day 0) minus the final weight (sampling day) of the minimally processed fruit in relation to the initial weight (day 0), and sensory evaluation of two defects that were found as significant sensory defects in previous assays with other orange varieties (Van de Velde et al., 2008), which were evaluated and qualified by consensus of the experimenters: dry surface and off-odors (usually, fermented odors) development using the following descriptive terms: none, mild, moderate, severe, and very severe. The physicochemical evaluations were done on the juice extracted from each sample. The content of four containers of minimally processed oranges, per treatment, were squeezed and filtered in the same way as the raw material. The juice was then used to perform the corresponding assays.

Ascorbic Acid (AA)

The 2,6-dichloroindophenol (DCIP) AOAC (1990) method was used to measure reduced ascorbic acid in accordance to Kevers et al. (2007), with some modifications. Each molecule of ascorbic acid converts a molecule of DCIP into a molecule of $DCIPH₂$, and that conversion can be monitored as a decrease in the absorbance at 520 nm. A standard curve was prepared using a series of ascorbic acid solutions. Samples of 5 ml were diluted 1*/*10 with a solution of metaphosphoric acid (3%) and acetic acid (8%), shaken manually and finally filtered. An amount of 700 µL of diluted samples or standard solutions were mixed with 900 µL of DCIP (0.1 mg*/*ml) and their absorbance was measured immediately. The blank value was determined with the aim of measuring the interference due to the sample color. For this purpose, 700 µL of diluted samples were mixed with $900 \mu L$ of distilled water. By using the blank optical density, the percentage of remaining DCIP was calculated as:

$$
\%remaining = ((A_{sample} - A_{blank})/A_{control})100,
$$
\n(1)

where $A_{control}$ is the absorbance of a solution prepared with 900 µl of 0.1 mg/ml DCIP mixed with 700 μ l of distilled water, A_{sample} is the sample absorbance, and *Ablank* is the blank absorbance. The calibration equation for ascorbic acid was determined by plotting the percentage of remaining DCIP versus the concentration of ascorbic acid in mg*/*ml. The samples were analyzed by duplicate, and results were expressed as mg*/*100 ml juice.

Antioxidant Activity (AEAC)

The antioxidant activity of the samples was estimated by determining the free-radical scavenging capacity evaluated with the stable radical DPPH∗, according to Sánchez-Moreno et al. (1998). For this purpose, the absorbance decrease of a methanol DPPH∗ solution at 517 nm in the presence of diluted samples (1*/*10) in acetone*/*water (80*/*20) was measured. The initial concentration of DPPH∗ was 0.03 g*/*L and the readings were taken after allowing the solution to stand for 30 min. The changes in absorbance were measured at a room temperature of about 25◦C. The percentage of remaining DPPH∗ was calculated as:

$$
\%remainingDPPH^* = (A_{sample}/A_{control})100,\tag{2}
$$

where *Acontrol* is the absorbance of 3.9 ml of DPPH[∗] solution mixed with a methanol volume equal to the sample volume used in the corresponding tube and *Asample* is the absorbance of 3.9 ml of DPPH[∗] solution mixed with 0.2–0.4 ml of sample. *IC*50(*sample*), the volume of diluted sample into 1 ml reaction needed to decrease by 50% the initial DPPH∗ concentration, was calculated from a simple regression analysis of the percentage of remaining DPPH∗ versus the juice concentration (ml juice*/*ml reaction) plot. The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) (Lim et al., 2007), using the following equation:

$$
AEAC = (IC_{50(AA)})/IC_{50(sample)})105,
$$
\n(3)

where *IC*_{50(*AA*)} was determined with a calibration curve of ascorbic acid solutions, and its value was 3.22 10−³ mgAA*/*ml solution. The determinations were made by duplicate and results were expressed as mg AA*/*100 ml juice.

Total Phenols Content (TPC)

The TPC was determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). The samples were diluted (1*/*10) with acetone*/*water (80:20). Aliquots of 0.4 ml of diluted samples were introduced in test tubes followed by 0.5 ml of Folin-Ciocalteu reagent and 2.5 ml of distilled water. The tubes were mixed using a vortex mixer and allowed to react for 3 min; then 1 ml of sodium carbonate (25%) and 5.6 ml of distilled water were added to it. The mixture was incubated for 30 min at room temperature before absorbance was measured at 760 nm. Reagent blanks were prepared by replacing the sample volume by distilled water. The total phenols content was performed by duplicate in each sample and results were expressed as gallic acid equivalents (mg GAE*/*100 ml juice).

Total Acidity and pH

Potentiometric titration was performed with a potentiometer (Oakton Instruments, Vernon Hills, IL) with pH scale, according to Rearte et al. (1987). A sample of 15 g of orange juice was titrated with a solution of 0.1 N sodium hydroxide until pH 8.1. The analysis was performed by duplicate and results were expressed as g citric acid*/*100 g juice. The pH values of juice samples were obtained with a pH meter (Twinphmeter Horiba B-213, Horiba Ltd., Kyoto, Japan) by duplicate.

Soluble Solids

The soluble solids content was determined by duplicate, in juices samples, using a hand-held digital refractometer (model Atago Pal-alpha, Atago, Bellevue, WA), with automatic temperature compensation. Results were expressed as ◦Brix.

Statistical Analysis

The experiment was a factorial design 2×5 (cut type and storage time) and subjected to analysis of variance using Statgraphics Plus® (version 5.1, Statpoint Technologies, Inc., Warrenton, VA) software. Means were separated by a Duncan multiple range test at 5% level of significance. Correlation analysis between AEAC and TPC or AA, were also done.

RESULTS AND DISCUSSION

Characterization of Plant Material

The initial characteristics of the oranges studied are shown in Table 1. The whole 'Valencia Late' oranges presented very good characteristics for being minimally processed: very few seeds and good balance of soluble solids

| Attribute | Mean value \pm SD ^z |
|--|----------------------------------|
| Unit weight of fruits (g) | 172.3 ± 10.1 |
| Percentage of peel (%) | 27.5 |
| Number of seeds per fruit | $0 - 3$ |
| Yield of minimal processing $(\%)$ | 52 |
| Percentage of juice m1/100 g of orange) | 59.6 ± 3.6 |
| Total acidity (g citric acid/100 g juice) | 0.5 ± 0.1 |
| рH | 3.9 ± 0.2 |
| Soluble solids (°Brix) | 12.5 ± 1.3 |
| Brix/acid ratio | 25.6 ± 4.6 |
| Ascorbic acid (mg/100 ml juice) | 37.5 ± 1.0 |
| Total phenols content (mg GAE/100 ml juice) | 92.9 ± 6.3 |
| Antioxidant capacity (mg AA/100 ml juice) | 45.8 ± 2.1 |

TABLE 1 Characterization of Sweet Oranges (*Citrus sinensis* L., Osbeck) cv. Valencia Late, for Minimal Processing

SD: Standard deviation.

vs. total acidity (reference value (Kader, 1994): brix*/*acid ratio *>*8). A previous study conducted with oranges 'Delta Seedless' and 'Valencia Late' from experimental crops in the province of Santa Fe (Argentina), showed values of brix*/*acid ratio of 9.6 and 7.8, respectively (Van de Velde et al., 2008). This showed that late season harvested commercial 'Valencia Late' oranges used in the present study had a better appealing flavor to be used as "ready to use."

Effect of Minimal Processing, Type of Cutting, and Storage Time

The proposed preparation process (on day 0) did not modify ($p > 0.05$) the quality of fresh fruit wedges and slices compared with whole oranges and showed no significant differences for ascorbic acid content, antioxidant capacity, and total phenols content (Fig. 1). The same behavior was observed for soluble solids, total acidity, pH, and brix*/*acid ratio (not shown).

Throughout the time analyzed, the ascorbic acid content was not significantly affected by type of cutting $(p > 0.05)$, as shown in Table 2. However, the effect of storage time was significant ($p \leq 0.05$), showing an ascorbic acid content decrease over time at $3°C$, particularly during the first three days for slices and five days for wedges (Table 2). The interaction of factors (time \times cut type) was not significant ($p > 0.05$). The percentage of ascorbic acid retention is presented in Figure 2. There was less than 30% loss after 10 days of storage at 3◦C. These results were in agreement with de Ancos et al. (2007) who reported a 24% loss in wedges and whole peeled oranges after 12 days storage at 4◦C and Rocha et al. (1995) who reported a 36% loss in ascorbic acid content in sliced oranges for 13 days at 4◦C. Ascorbic acid degradation has been associated with specific enzymes (cytochrome oxidase, ascorbic acid oxidase, and peroxidase) and the possibility of non-enzymatic degradation reactions of ascorbic acid in the presence of oxygen (Rocha et al., 1995).

The total phenol content did not change significantly either with cut type or with the storage time (Table 2), and its average value was $95.2 \pm$ 5.8 mg GAE*/*100 ml juice. Kevers et al. (2007) reported a transient decrease of these compounds (−76% on day 2) in citrus stored at room temperature for 15 days. On the other hand, Artés-Hernández et al. (2007) found a gradual decrease in the total phenol content in minimally processed lemons with different cut types (wedges, slices, $\frac{1}{2}$ and $\frac{1}{4}$ slices) stored at different refrigeration temperatures for 10 days. That decrease was independent on storage temperature, but it was dependent on cut type. They found a correlation between the cut type and the quantity of phenolic compounds at the end of shelf life. The lower decrease in phenolic content was found in $\frac{1}{4}$ slices in which the greatest wounding-induced stress occurred. A possible explanation for this behavior was the fact that phenolic compounds were involved

FIGURE 1 Antioxidant capacity (AEAC), ascorbic acid content (AA), and total phenols content (TPC) in minimally processed sweet oranges, whole (W), in slices (S), and wedges (We), immediately after processing. Each value is the average of four samples. Vertical bars indicate standard deviations.

in many interactions of plants in response to factors of biotic and abiotic stresses; it could be the reason for the increase or reduced decrease in total phenol content in the lemons with higher cut or injury degree. According to Reyes et al. (2007), wounding of tissue leads to an increased synthesis of phenolic compounds associated with an increased activity of the enzyme phenylalanine ammonia lyase (PAL). This behavior was observed only in those plants with low levels of ascorbic acid, where it was quickly consumed by the neutralization of reactive oxygen species (ROS) produced by

| Time (days) | AA $(mgAA/100 \text{ ml }$ juice) | | AEAC $(mgAA/100 \text{ ml }$ juice) | | TPC $(mgGAE/100 \text{ ml }$ juice) | |
|----------------|--------------------------------------|---------|--|--------|---|--------|
| | Slices ^z | Wedges | Slices | Wedges | Slices | Wedges |
| $\overline{0}$ | $34.2\text{A}a$ ^y | 36.4Aa | 47.1Aa | 51.7Aa | 95.9Aa | 90.4Aa |
| 3 | 32.9 Aab | 32.8Aab | 41.9Ab | 40.4Ab | 94.4Aa | 97.4Aa |
| 5 | 31.0Ab | 28.8Ac | 38.3Ac | 36.2Ac | 97.4Aa | 91.6Aa |
| | 30.6Ab | 29.1Ac | 39.5Ac | 35.2Ac | 92.9Aa | 90.5Aa |
| 10 | 30.6Ab | 28.3Ac | 35.6Ac | 34.8Ac | 103.6Aa | 97.9Aa |

TABLE 2 Changes in Ascorbic Acid (AA), Antioxidant Capacity (AEAC) and Total Phenol Content (TPC) in Minimally Processed Sweet Oranges for Both Types of Cutting, During Storage at 3◦C

*z*Values represent the mean (*n* = 8).
*y*Values in the same column with different lowercase letters are significantly different (*p* \leq 0.05) by Duncan's test. Values in the same row for each attribute with different capital letters are significantly different ($p \leq 0.05$) by Duncan's test.

FIGURE 2 Percentage of ascorbic acid (AA) retention in minimally processed sweet oranges for both types of cutting, during storage at 3◦C. Each value is the average of eight samples. Vertical bars indicate standard deviations.

lesions on the tissue, and phenols are possibly synthesized to partly control ROS.

The antioxidant capacity was significantly affected by storage time ($p \leq 0.05$), but not by type of cutting ($p \geq 0.05$) (Table 2). It decreased from day 0 to day 5, and then it remained constant until day 10. Del Caro et al. (2004) studied the changes in the nutritional and health-giving properties of different species and cultivars of citrus fruits processed as wedges and juices at 4◦C for 12 or 15 days. They found that antioxidant capacity increased significantly in *Salustiana* orange segments, decreased in 'Minneola' tangelo segments, and remained constant in other samples. Artés-Hernández et al. (2007) found that for lemons the antioxidant activity remained constant or slightly decreased in all cut types and storage temperatures tested. de Ancos et al. (2007) reported a trend of increasing antioxidant capacity during storage at 4◦C until day 15 in minimally processed orange wedges. Plaza et al. (2011) found that at the end of refrigerated storage, the antioxidant activity remained stable with regard to the initial values in each sample. In addition, there were no significant changes among samples (whole, peeled, and segments). Therefore, it could be concluded that the antioxidant activity in oranges would depend on cultivar, impact of processing, and storage conditions. The antioxidant capacity correlated with the ascorbic acid content both in slices ($R^2 = 0.87$) and in wedges ($R^2 = 0.95$). These results clearly indicated that ascorbic acid was the main antioxidant compound found in citrus fruits and it would make the major contribution to the total antioxidant capacity. According to Gardner et al. (2000), vitamin C was found to account for 65 to 100% of the antioxidant potential of beverages derived from citrus fruit. Similarly, Sánchez-Moreno et al. (2003) observed that the major antioxidant in all orange juices tested was vitamin C (about 99.5%), and Del Caro et al. (2004) found that the correlation between antioxidant capacity values and ascorbic acid content of citrus fruit segments showed a fairly high value. On the other hand, in the present work, the correlation between total phenols content and total antioxidant capacity was not good either for slices ($R^2 = 0.31$) or wedges ($R^2 = 0.11$), indicating a lower contribution of phenolic compounds to the total antioxidant capacity. These results were in agreement with those of Sánchez-Moreno et al. (2003), Del Caro et al. (2004), de Ancos et al. (2007), and Artés-Hernández et al. (2007), again confirming that ascorbic acid is the main antioxidant in fruits of the *Citrus* genus.

There were no significant changes ($p > 0.05$) in soluble solids content until day 10 for both types of cutting (Table 3). Its average value was 12.7 \pm 0.1 °Brix. These results were consistent with those found by Rocha et al. (1995) and Rapisarda et al. (2006) for other minimally processed orange varieties stored at 4◦C.

Total acidity and pH were significantly affected ($p \leq 0.05$) by storage time (Table 3). A slight decrease in pH value was observed on day 3 of storage and remained constant for the rest of the storage. A decrease in

| Time (days) | SS $(^{\circ}Brix)$ | | рH | | TA $(mg CA/100 ml)$ juice) | |
|-------------|---------------------|--------|----------|--------|----------------------------|--------|
| | Slices ^z | Wedges | Slices | Wedges | Slices | Wedges |
| Ω | 12.9Aa ^y | 12.9Aa | 4.2 Aa | 4.3Aa | 0.6Aa | 0.6Aa |
| 3 | 12.8Aa | 12.8Aa | 4.0Ab | 3.8Ab | 0.5Ab | 0.5Ab |
| 5 | 12.3Aa | 13.0Aa | 3.9Ab | 4.0Ab | 0.4Ab | 0.5Ab |
| | 12.8Aa | 12.6Aa | 3.9Ab | 3.8Ab | 0.5Ab | 0.4Ab |
| 10 | 12.4Aa | 12.4Aa | 4.0Ab | 3.8Ab | 0.4Ab | 0.5Ab |

TABLE 3 Changes in Soluble Solids (SS), pH, and Total Acidity (TA) in Minimally Processed Sweet Oranges for Both Types of Cutting During Storage at 3◦C

^zValues represent the mean (*n* = 8).
^yValues in the same column with different lowercase letters are significantly different (*p* ≤ 0.05) by Duncan's test. Values in the same row for each attribute with different capital letters are significantly different ($p \leq 0.05$) by Duncan's test.

FIGURE 3 Percentages of weight loss (%WL) and exudates (%E) in minimally processed sweet oranges, for slices (S) and wedges (We), during storage at 3◦C. Each value is the average of four samples. Vertical bars indicate standard deviations.

the acidity was observed on day 3 and then remained unchanged for the rest of the storage time. This decrease might be due to increased respiration following the minimal processing. Similar results were found by Karacay and Ayhan (2010) in orange segments packaged under different types of modified atmospheres.

The storage time and type of cutting were significant factors ($p \leq$ 0.05) for the percentage of weight loss and the percentage of exudates (Fig. 3). The percentage of weight loss reflects the liquid weight on the bottom of the tray plus the condensed water on top of it. This percentage was higher in slices (1.85%) than wedges (0.65%) at day 3. From day 5 to the end of experiment, weight loss of both slices and wedges remained constant throughout storage (mean value: 2.05%). The percentage of exudates presents the same behavior, higher in slices (1.50%) than wedges (0.60%) at day 3, and then remained constant until the end of the experiment (mean value: 1.73%). These results could be explained by the greater degree of wounding in the cut type "slices" compared with "wedges," resulting in a greater leakage of liquids from the cells.

The sensory evaluation focused on off-odors and dry surface development indicated the same intensity of defects both in wedges as in slices. The off-odors development was considered moderate after day 7 at 3◦C for both types of cutting. Moreover, the dry surface defect that had been present in other orange cultivars (Van de Velde et al., 2008) was absent until day 10 of storage for both types of cutting studied here.

CONCLUSIONS

The minimal processing proposed in this study did not cause quality losses compared to the whole fruit in the ascorbic acid content, antioxidant capacity, total phenolic content, and soluble solids. Although sensory evaluation showed no differences between cut types in the development of off-odors and dry surface defects, wedges minimized the formation of exudates and weight loss. The wedges and slices did not show changes in total phenols content during storage at 3◦C for 10 days. However, they showed changes in ascorbic acid content and antioxidant capacity. Both ascorbic acid content and antioxidant capacity decreased no more than 30% in this period. The antioxidant capacity presented a high correlation with the ascorbic acid content, which would indicate the great contribution of ascorbic acid to the total antioxidant capacity in citrus fruits. The total phenols were constant during the refrigerated storage and showed no good correlation with antioxidant capacity, indicating the minor contribution to the healthy potential of citrus. These results are relevant because they show that the availability of this bioactive compound is preserved along the potential shelf life of the product.

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