衝撃ストレスが収穫後キャベツの生理的・化学的特性に及ぼす影響

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<td>Thammawong, M.  兼田, 朋子  中村, 宣貴  吉田, 誠  曽我, 綾香  椎名武夫,</td>
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Influence of Impact Stress on the Postharvest Physiological and Chemical Properties of Cabbage Heads

THAMMAWONG Manasikan*1, KANETA Tomoko*1, NAKAMURA Nobutaka*1, YOSHIDA Makoto*2, SOGA Ayaka*2 and SHIINA Takeo*1,‡

*1 National Food Research Institute, National Agriculture and Food Research Organization, 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642
*2 Kanagawa Agricultural Technology Center, 1617, Kamikitsusawa, Hiratsuka, Kanagawa 259-1204

Because the mechanical damage caused by postharvest handling (including bulk storage and transport) plays an important role in the yield and the postharvest quality of fresh produce, the effect of postharvest impact stress on the physiological and chemical properties of cabbage (Brassica oleracea var. Capitata L.) was investigated in this study. To simulate the impact stress occurrence during the bulk handling practice, cabbage picked at commercial harvest stage was dropped from different heights (10, 20, 40, and 80cm) onto a flat, rigid concrete floor. The effects of the dropping treatment on the respiration rate and on the content of various sugars (sucrose, glucose, and fructose) were evaluated during storage at 20°C for 6 days. Shortly after the dropping treatment, an increased rate of respiration correlated with a higher dropping height. However, a decreased respiration rate was observed after one day of storage. The sugar content of all samples changed slightly at days 1 and 3. At day 6, cabbages dropped from 80 and 40cm showed the lowest content of fructose and total sugar (the sum of sucrose, glucose, and fructose). The major effects of impact stress on cabbage heads include increases in the respiration rate and a loss of sugar content. In addition, tests of controlled atmosphere (CA) treatment on dropped cabbages showed that CA likely inhibited the severe damage caused by impact stress on cabbages because it reduced the rise in respiration rate and slowed the loss of sugar content in dropped cabbages during storage at 20°C. Overall, impact stress obviously affects the postharvest physicochemical properties of cabbage just after impact occurrence (respiration rate) and during storage (sugar content). These changes are also influenced by the strength of the stress level (height of dropping treatment) and the time period after the application of the stress treatment. Rapid and appropriate postharvest handling and storage management, therefore, will delay the onset of deterioration of cabbage quality and extend its shelf life.

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Key words : cabbage, controlled atmosphere (CA), dropping treatment, HPLC analysis, impact stress, respiration rate, sugar content
キャベツ, 修正空気, 落下処理, 高速液体クロマトグラフ分析, 衝撃ストレス, 呼吸速度, 糖含量

Mechanical damage (bruising, wounding, and cracking) is considered a type of stress that occurs during postharvest handling. This stress is accompanied by physiological, biochemical, and morphological changes that affect the postharvest yield and quality of fruits and vegetables and cause rejection by wholesale and retail markets. The postharvest damage of fresh produce is usually caused by one or more types of stress: impact, compression, and vibration. Impact stress is mechanical/dynamic stress, which is a transitory change, occurring through a sudden acceleration or deceleration causing a high dissipation of energy and subsequent damage to the produce. It occurs at varying intensities during packing, transport, and storage handling. Moreover, there is a high probability of impact stress when a large amount of produce is pooled and transported together in one large bulk container; this stress will increase quality deterioration and consumer rejection of fresh produce.

† These authors contributed equally to this work.
‡ Corresponding author, E-mail address: shiina@affrc.go.jp
produce.

Bulk containers are generally used for storing and transporting goods from one location to another. In Japan, the newly developed large size bulk container (cardboard body with plastic base and cover, outside dimensions approx. L 113cm × D 115cm × H 100cm) has been proposed for fresh fruits and vegetables distribution. This returnable and reusable large size bulk container offers not only reduced cost, but also abated emission of greenhouse gases\(^7\). However, with using this large bulk container in the field or at the packing house, cabbage heads are subject to impact stress / damage when dropped into the container, resulting in wounded outer leaves. Additionally, the physical damage and quality deterioration of fruits and vegetables transported by bulk container including cabbages has not been well clarified.

Cabbage (Brassica oleracea var. Capitata L.) is an economically important crop grown and consumed worldwide. Cabbages are generally harvested by hand to avoid mechanical damage from machine harvesting. After harvesting, they are packed and transported to marketplaces or are placed into bulk containers and stored in cold storage for future packing and distribution\(^9\). During handling and distribution, loss of quality and yield caused by impact stress is likely due to improper postharvest handling and management of bulk storage and transportation.

A study of the effect of mechanical stress in cabbage on the cracking of leaf layers has been conducted\(^7\). The effect of wounding / cutting on volatile compounds has also been investigated\(^9, 10\). However, little is known about the stress response mechanism in cabbage, and there are few reports investigating the effect of mechanical stresses (impact, compression, and vibration) on the physiological and biochemical changes in cabbage heads, either directly after receiving stress or during long-term storage.

This study, therefore, aimed to understand the effects of dropping treatment (the impact stress simulation during the bulk handling practice) on the postharvest quality of cabbage heads during storage at 20°C. After the dropping treatment, we investigated changes in the respiration rate and the individual sugar content at days 0, 1, 3, and 6. In addition, because there are few studies addressing the effect of impact stress on the physicochemical properties of cabbage heads under controlled-atmosphere (CA) storage, the respiration rate of dropped cabbage samples under air or CA conditions were continuously monitored in this work, and after 6 days of storage, the sugar contents of cabbage samples were analyzed.

Materials and Methods

1. Plant materials

One hundred and twelve heads of ‘Hamamisaki’ cabbage (Brassica oleracea var. Capitata L.) were obtained from a commercial farm in Miura City (Kanagawa prefecture, Japan) on 15 Nov. 2010. They were harvested and transported by car with air conditioning (20°C, approx. 2h) to the laboratory at the National Food Research Institute, Tsukuba, Ibaraki, Japan. After arrival, cabbages were selected for experimentation based on uniformity of shape and size. The average height (from cut end to head top) and equatorial diameter of the cabbage head samples were 140.3 ± 7.1mm and 174.3 ± 8.0mm, respectively. The selected cabbages (81 heads) were transferred to a storage room at a constant temperature of 20°C overnight to equilibrate their temperature. After this prestorage, the cabbage samples were then used for the following dropping and storage experiments.

2. Dropping treatment and storage conditions

The harvested cabbages were treated with different degrees of impact by letting the cabbage head fall from the heights of 80cm, 40cm, 20cm, and 10cm. Each individual cabbage head was dropped 3 times from the same height onto a flat, rigid concrete floor, with the cut end (core) of the cabbage facing up (Fig. 1). Three replicates (1 head = 1 replicate) were analyzed in this study, with a completely randomized design. The highest height of the dropping treatment (80cm) was designed based on the height of bulk container (100cm) and the size of cabbage (approx. 20cm). Additionally, the leaf pack side of cabbage was dropped onto a rigid floor because it is likely to be the most sensitive part of the head to the impact stress. The injury symptoms of cabbage leaves caused by the dropping treatment are shown in Fig. 2. Slight browning symptom of wounded part was observed during the storage; however it seemed not to affect the rest of the leaf area (data not shown).

To investigate the changes of chemical
Fig. 1 Schematic illustration of the dropping treatment of cabbage heads at 80, 40, 20 and 10 cm

Fig. 2 Injuries caused by dropping treatment from the height of 80 cm: the first layer (A) and the fourth layer (B) of the head leaf pack of cabbage

components during storage at 20°C and to allow gas exchange and prevent water loss, the cabbages dropped from the four levels and the control cabbages (total 45 heads) were kept in closed and unsealed polyethylene (PE) bags at 20°C for analysis at days 1, 3 and 6.

For sample collection, each cabbage head was individually cut at the equatorial line (Fig. 3), because the impact injury was observed not only in the first leaf layer but also in other layers of head leaf pack (Fig. 2). The leaf pack on the opposite side of the core was sampled using a cork borer (φ 2 cm). The cabbage leaf disks were randomly mixed, weighed, and immediately frozen in liquid nitrogen. The frozen samples were then kept at −40°C until the analysis of the sugar content was performed.

3. Respiration measurement

The fifteen heads of cabbage (four sets of dropped cabbages and one control set, each set containing 3 heads) were used for respiration analysis at days 0, 1, 3, and 6. Each cabbage head was weighed and sealed in a 12.1 ℓ plastic container, and 1 ml of headspace gas was injected into a gas chromatograph (GC 6-AM: Shimadzu, Kyoto, Japan) equipped with a ZY-1 column (Shimadzu, Japan) held at 70°C and a thermal conductivity detector held at 80°C. Helium was used as the carrier gas.

4. CA treatment

Six chambers (10 mm thick acrylic plate, 280 mm × 280 mm × 385 mm) were used in this study: three chambers for air storage and three chambers for CA storage. After the dropping treatment, two groups of three treatments (control cabbage, 80-cm dropped, and 20-cm dropped, each treatment containing 3 heads) were selected. One group was stored in air chambers and another group was stored in CA chambers. The respiration measurement of all samples was conducted with a flow-through system. The inlet gas composition with a constant mass flow control of the air chamber was 21% O₂ + 79% N₂ + 0% CO₂ and that of the CA chamber was 2.5% O₂ + 4.5% CO₂ balanced with N₂. Six chambers were individually linked with a gas chromatograph.
fraction. The supernatant was collected and the pellets were rehomogenized twice with 10 ml of 80% (v/v) ethanol at 10,000 rpm for 1 min (NS-51 Physcon homogenizer, Microtech Nition, Chiba, Japan). The homogenate was centrifuged at 2,525 g, for 10 min (Biofuge primo, SORVALL® Osaka, Japan) to yield an ethanol-soluble fraction. The supernatant was collected and the pellets were rehomogenized twice with 10 ml of 80% (v/v) ethanol at 10,000 rpm for 1 min. After centrifugation, the three ethanol soluble fractions were pooled together and the volume was adjusted to 45 ml with 80% (v/v) ethanol. Nine milliliters of each sample was evaporated to dryness under N2 at 70°C and then redissolved in 1 ml of Milli-Q water (Nihon Millipore, Ltd., Japan). The concentration factor of the final sample solution in this study was therefore 1-fold (5 g/45 ml extraction x 9 ml/1 ml [at purification] = 1.0). The soluble fraction was centrifuged at 1,000 g, for 5 min (Biofuge primo, SORVALL®) to produce a clear solution and then filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter (Advantec, Toyo Roshi Kaisha, Ltd., Japan).

Standard solutions of sucrose, glucose, and fructose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). A stock standard solution of the three sugars mixed (30.0 mg · ml⁻¹ each) was prepared, and the standard mixture was diluted in Milli-Q water to the desired concentration for standard curve calibration (0.0, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, and 30.0 mg · ml⁻¹). A high-performance liquid chromatography (HPLC) system (LC-10 AVP; Shimadzu) was used for sugar determination. Degassed Milli-Q water at 0.5 ml · min⁻¹ and 60°C was used as the mobile phase. A refractive index detector (RID-10A; Shimadzu) was used to quantify the sugar content following separation with a Shim-pack SCR-101N column (Shimadzu).

6. Data analysis

An analysis of variance with a completely randomized design using dropping levels or days of storage as factors was performed using SPSS (SPSS, Chicago, IL). Tukey’s multiple-range test was used to test for a significant difference at the 95% confidence level of each variable.

Results

1. Effect of dropping treatment on the respiration rate of cabbages

The largest change in respiration rate (mg CO₂·kg⁻¹·h⁻¹) was observed at day 0 (measured immediately after the dropping treatment). The highest respiration rate was found in the 80-cm dropped sample (ca. 70 mg·kg⁻¹·h⁻¹), and lower rates were observed in the 40-cm dropped, 20-cm dropped, and control samples, respectively (Fig. 1). In addition, a gradual decrease in the respiration rate of all cabbages (treated and control) was observed during storage at 20°C for 6 days.

2. Effect of dropping treatment on the sugar content of cabbages

The changes in total sugar, sucrose, glucose, and fructose during storage are shown in Fig. 5. The total sugar content slightly decreased during the 6 days of storage. The differences in the total sugar levels were small at day 1 and day 3. However, a significant loss of sugar was observed in the 40-cm dropped cabbages at day 6 (P < 0.05, Fig. 5A). The sucrose content of the cabbage heads in this group was 0.8~1.1 mg · g⁻¹ fresh weight (FW), the lowest sugar content in this research. A small change in sucrose content was observed during the 6 days of storage, but a significant difference was not found among the various treatments (Fig. 5B). The glucose content also changed slightly during storage. At day 6, there was a difference in the glucose content among the dropping treatments, with the lowest
was observed at day 6 (Fig. 5D). For the fructose content, the highest content of 15 mg·g⁻¹ FW was observed at day 0, and it gradually decreased during storage. The most obvious change in fructose content among the different treatments was observed at day 6 (Fig. 5D), with a significant loss in the 80-cm and 40-cm dropped samples. In addition, there was a small change in the fructose content in the 10-cm dropped samples that was not significantly different from the content in the control cabbages.

3. Changes in respiration rate and sugar content of dropped cabbages stored under CA conditions

The respiration rate (mg CO₂·kg⁻¹·h⁻¹) of cabbage samples under air and CA conditions was monitored for 150 h (Fig. 6). Under air conditions, the respiration rate of all of the samples increased during the first 10 h of measurement, with the highest value observed in the 80-cm dropped sample. However, in contrast to air conditions, the respiration rate of the 80-cm dropped sample stored under CA conditions was approximately 50% lower than that of samples stored under air conditions (Fig. 6). Moreover, the respiration rate of all cabbage samples gradually decreased, reaching below 20 mg CO₂·kg⁻¹·h⁻¹ after 50 h. Unfortunately, the respiration data of the cabbage stored under CA were shorter (112 h) than that of air storage because of the instability of the gas regulating system (Fig. 6B). The concentration of the inlet carbon dioxide also revealed the instability of the gas conditions at 112–130 h of investigation (Fig. 6C), resulting in difficulties in calculating the respiration rate for this period.

Table 1 shows the content of the different types of sugars in cabbage heads stored at 20°C for 6 days under air or CA conditions. No significant difference was found in the glucose content of any of the samples. However, although there was no significant difference between the air and CA conditions, a loss of fructose and total sugar content was observed at day 6 of storage, with the lowest content in the 80-cm dropped samples stored under air conditions. Sucrose was the least abundant sugar among the three individual sugars monitored in this experiment.
Mechanical damage causes cellular changes in fresh produce, affecting qualities such as flavor, appearance, and physiological properties\(^{20-23}\). Plant organs subjected to mechanical stress also induce changes in respiration rate and ethylene production\(^{20,21}\). Mechanical stress, therefore, is one of the most important factors leading to quality deterioration and reducing the shelf life of fresh produce. For lengthy storage of fruits and vegetables, controlled and/or modified atmosphere storage has been reported as a useful tool for shelf life extension, especially for those that deteriorate rapidly. Our present work shows the effect of impact stress on the physiological and biochemical changes in cabbage stored at 20°C (in air and CA conditions).

Under ambient air storage of cabbage, the respiration rate of cabbage dropped from a height of 80cm was higher than another dropping treatments. With the lowest respiration rate observed in control cabbages (Fig. 4). For respiration analysis during storage at 20°C for 6 days, the results showed a gradual decrease of respiration rates in all samples. From the results, it can be hypothesized that the sudden impact stress resulting from the dropping treatment immediately induces a higher respiration rate in the cabbage head after it receives a stress signal. Although the mechanism of the dropping effect on the physiological and biochemical properties is still not clear, this study indicated that an increase in respiration rate is one of the sensitive internal responses of cabbage to impact

Table 1 Changes in the individual sugar content (mg·g\(^{-1}\) FW) of control and dropped cabbages during storage under air or CA conditions at 20°C for 6 days

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Day</th>
<th>Air Control</th>
<th>80cm</th>
<th>20cm</th>
<th>CA Control</th>
<th>80cm</th>
<th>20cm</th>
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<tr>
<td>Sucrose</td>
<td>0</td>
<td>0.77 ± 0.28</td>
<td>0.60 ± 0.06</td>
<td>0.77 ± 0.06</td>
<td>1.54 ± 0.38</td>
<td>1.32 ± 0.08</td>
<td>1.70 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.87 ± 0.21</td>
<td>a</td>
<td>a</td>
<td>1.12 ± 0.06 c</td>
<td>1.32 ± 0.08 b</td>
<td>1.70 ± 0.09 c</td>
</tr>
<tr>
<td>Glucose</td>
<td>0</td>
<td>16.04 ± 0.51</td>
<td>a</td>
<td>a</td>
<td>15.43 ± 0.28 a</td>
<td>16.16 ± 1.50 a</td>
<td>16.09 ± 0.83 a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15.45 ± 0.78</td>
<td>a</td>
<td>a</td>
<td>14.19 ± 0.33 a</td>
<td>15.43 ± 0.28 a</td>
<td>16.06 ± 1.12 a</td>
</tr>
<tr>
<td>Fructose</td>
<td>0</td>
<td>14.91 ± 0.35</td>
<td>a</td>
<td>a</td>
<td>10.37 ± 0.96 b</td>
<td>11.75 ± 0.07 b</td>
<td>12.23 ± 1.86 b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12.18 ± 0.64</td>
<td>b</td>
<td>b</td>
<td>10.37 ± 0.96 b</td>
<td>11.75 ± 0.07 b</td>
<td>12.94 ± 0.59 b</td>
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\(*=\)Different letters (a, b, and c) indicate a significant difference among treatments compared with Day 0 at \(P < 0.05\), as determined by Tukey’s multiple-range tests.

Each value is the mean of three replicates ± SD.
stress. Cabbage may require a large amount of energy to respond to a sudden impact stress. A high respiration rate can supply energy for cellular metabolism. Additionally, large increases in carbon dioxide and ethylene production have been reported as a response to mechanical stresses, such as wounding in fruits and vegetables. Increased respiration has been correlated to the enhanced synthesis of enzymes involved in the respiratory pathway and to a transient increase in aerobic respiration in fresh-cut carrots.

With respect to the biochemical properties of fresh produce, many previous studies have described changes in soluble solids induced by mechanical damage. Our study also demonstrates the effect of impact stress on changes in sugar content. The results revealed that the dropping treatment induced the loss of fructose and the total sugar content of cabbage heads during storage at 20°C, especially at day 6 of the investigation. This indicates a change in the chemical components in dropped cabbage in response to impact stress and is likely caused by a metabolic response following the rise of the respiration rate. Additionally, according to the close relationship between sugar content and cellular respiratory metabolism, a decrease in soluble solids might be associated with the use of sugars as respiration substrates. Nevertheless, the dropping treatment from a height of 10 cm did not cause changes in individual sugar content during the 6 days of the investigation. For future applications in agricultural research and postharvest handling, further study of various types of mechanical stress and acceptable levels of mechanical damage in cabbage and other fresh produce should be considered.

Cabbage is perhaps the most common vegetable stored under CA conditions, under the recommended storage levels of 2% to 3% O2 and 4% to 5% CO2. In this study, CA treatment reduced the rise in the respiration rate of dropped cabbages during storage at 20°C (Fig. 6). For most vegetable products, when the external oxygen pressure is low, the respiration activity is decreased, a change attributed to a reduction of oxidase activities. Low O2 levels can also suppress the genes and enzymes that are associated with maturation and senescence, such as acid invertase, cellulase and polygalacturonase. The decrease in the respiration rate of dropped cabbage under CA treatment might relate to the inhibitory activities described above. Moreover, CA conditions of 2.5% O2 and 4.5% CO2 appear to effectively inhibit physiological changes and to extend the shelf life of cabbage under room temperature storage. However, it is important to consider that the degree of impact stress levels will likely influence the efficiency of CA storage.

In general, fruits and vegetables stored under modified or controlled atmospheres maintain high values of soluble sugars and organic acids. Increases in sugar content were also observed in previous studies of various fresh produce. Although there was no significant difference between air and CA conditions in this study, CA treatment seemed to maintain glucose content during storage and to slow the loss of fructose content. Moreover, at day 6 of the investigation, an increase of sucrose (ca. 2-fold) was found in all samples receiving CA treatment (Table 1). The small change in sugar content might also be due to the low respiration of cabbage under CA storage conditions (Fig. 6). Additionally, because the most pronounced increase in respiration rate in cabbage occurs immediately after the impact stress and decreases gradually thereafter, the management of postharvest handling, including both the storage and transportation stages, should receive more attention and maintenance in a controlled and/or modified atmosphere should immediately follow field harvesting to reduce injury to the cabbage by mechanical stress.

In conclusion, the application of impact stress by dropping treatment to cabbages caused an increase in respiration rate and a loss of sugar content. The strength of the stress level and the time period after the application of stress also play a role in cellular changes in the cabbage. In addition, CA treatment seemed to inhibit physiological and chemical changes during 150 h of storage. This technique may reduce the severe damage caused by impact stress in cabbages.

Postharvest impact stress induces quality deterioration of cabbages. However, quick and appropriate manipulation of postharvest handling can maintain quality and reduce losses of cabbage yield. Moreover, as the general storage and transport of cabbage is conducted in bulk, further studies of the effect of mechanical stress on the postharvest quality of cabbage during bulk handling and
distribution should be considered.

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References

衝撃ストレスが収穫後キャベツの生理的・化学的特性に及ぼす影響

タンマウォン マナスィカン"1・兼田朋子"1・中村宣貴"1
吉田 誠"2・曾我彼香"2・植名武夫"2

＊1 農研機構食品総合研究所
（〒305-8642 茨城県つくば市観音台 2-1-12）
＊2 神奈川県農業技術センター
（〒259-1204 神奈川県平塚市上吉沢1617）

青果物の収穫後の取扱における物理的損傷は、ロスや品質低下に大きな影響を及ぼす。そこで、本研究では、パルクでの荷扱いを想定し、物理的ストレスのうちの落下衝撃が、キャベツの生理的、化学的特性に及ぼす影響について検討した。通常収穫したキャベツを、10、20、40、80cmの高さから水平コンクリート床に自由落下させ、その後、20℃に6日間保存し、保存中の呼吸速度、糖含量（スクロース、グルコース、フラクトース、およびそれらの合計としての全糖）を経時的に測定した。キャベツの呼吸速度は、落下処理直後に上昇し、その程度は、落下高さが大きいほど顕著であった。その後、呼吸速度は低下し、落下処理1日後には、対照区（落下処理無し）のそれぞれに、有意的な差はみられなくなった。フラクトースと全糖は、保存1、3日後にやや低下し、6日後には顕著な低下がみられ、80cm、40cmから落下処理したキャベツで最も低い値を示した。すなわち、落下処理による糖の減少が、化学的、生物的影響として、呼吸速度の上昇と、糖含量の低下があることが示された。一方、落下処理後に、20℃、CA環境下に保存したキャベツに比べ、呼吸速度の上昇と糖含量の減少が抑制されたことから、CA環境によって落下ストレスによる生化学的損傷が低減されるものと考えられた。以上の結果から、収穫後のキャベツにおいて、落下処理は、処理直後の呼吸速度の上昇を、保存中の糖含量の減少を引き起こすこと、その影響が落下高さに依存すること、さらに、CA環境を含めた適切な環境の維持によりストレスの軽減と品質劣化の抑制が可能であることが示された。

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