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論　文　名	Study on effects of hot water treatment in red sweet pepper fruit during low temperature storage (低温貯蔵中の赤ピーマン果実における温湯処理の効果に関する研究)	
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論文要旨

Red sweet pepper (*Capsicum annuum* L.) is grown in tropical and subtropical regions as an important horticultural product in terms of the global economy. Red sweet pepper fruit has high nutritional value (a good source of vitamin A and C) and contains bioactive materials such as phenolic compounds and carotenoids. However, red sweet pepper fruit is a perishable commodity with fruit ripening when stored at ambient temperature, resulting in short shelf life after harvest. Thus, a suitable storage method is required to ensure the quality of red sweet pepper fruit.

Low temperature storage maintains the quality of horticultural products and extends storage duration. The shelf life of red sweet pepper fruits is limited as a result of decay, loss of water during storage, and sensitivity to cold stress. The optimal storage temperature for red sweet pepper fruit was reported to be 12 °C, with chilling injury (CI) often occurring below this temperature.

Many technologies have been developed to mitigate CI of horticultural products, including physical treatments such as controlled or modified atmosphere, irradiation, and heat treatment, and chemical treatments using plant hormones and natural elicitors. Heat treatment is a safe and environment-friendly method and that is effective postharvest treatment. Horticultural products are treated previous to low temperature storage utilizing hot water, hot air, or vapor heat. Water is a more efficient heat transfer medium than air and is preferred for most heat treatment applications. Thus,

hot water treatment is expected the beneficial effect for mitigation of CI, enhancing chilling tolerance by improving antioxidant responses.

However, little information is available concerning oxidative stress and adaptive responses in red sweet pepper fruit to chilling stress under hot water treatment. This study investigated the effects of hot water treatment on the level of oxidative stress and CI incidence during storage at low temperature, and discussed the possible mechanisms leading to the induction of chilling tolerance.

Chapter 1: Effects of chilling injury and quality of red sweet pepper fruit subjected to different temperatures and times of hot water treatment

Temperature and treatment time are important factors for hot water treatment. The physiological response of fruit under low temperature stress varies with temperature and duration of hot water treatment. Hot water treatment at 45 °C for 1, 5 and 10 min and 55 °C for 1, 3 and 5 min were applied for fruits stored at 10 °C for 4 weeks. Hot water treatment at 55 °C caused heat damage, while heat damage was also observed in fruit treated at 45 °C for 10 min. No decay was recorded in fruits treated at 45 and 55 °C for 1 min. Weight loss in fruits treated at 45 and 55 °C for 1 min was lower than for the other treatments. Fruit color (L^*, a^* and b^* values) was not affected by hot water treatment, with no significant difference in all fruits during storage. Hot water treatment at 45 and 55 °C for 1 min maintained firmness throughout the storage time. Fruits treated at 45 and 55 °C for 1 min showed a delay in CI development with lower CI incidence. In week 4, CI in fruits treated at 45 and 55 °C for 1 min were slight but severe in untreated fruits and fruits exposed to longer heating time. During low temperature storage, fruits treated at 45 and 55 °C for 1 min had lower electrolyte leakage and malondialdehyde (MDA) levels reflecting the lower degree and incidence of CI. Total ascorbate (AsA) levels in fruit treated at 45 and 55 °C for 1 min gradually decreased during storage but were maintained at a higher level than in fruits exposed to prolonged heating treatments and untreated fruits.

Hot water treatment at 45 °C for 1 min mitigated CI and decay, maintaining fruit color, firmness, and total ascorbate level. Therefore, hot water treatment at 45 °C for 1 min was chosen to investigate the relation between CI incidence and antioxidant system in red sweet pepper fruit during low temperature storage.

Chapter 2: Antioxidant responses to hot water treatment of red sweet pepper fruit during low temperature storage

Fruits were treated with hot water at 45 °C for 1 min and stored at 5 °C for 4 weeks. Hot water treatment delayed CI development and incidence during storage. At the end of the storage period, CI was only slightly developed in the treated fruit but was severe

in untreated fruit (control). Treated fruit had lower MDA and hydrogen peroxide (H_2O_2) levels than the control, indicating that hot water treatment enhanced the scavenging of reactive oxygen species (ROS) and inhibited membrane lipid peroxidation. Plants react to oxidative stress by non-enzymatic and enzymatic systems that sustain the equilibrium between ROS generating and scavenging. These systems include both antioxidants of ascorbate and glutathione (GSH) and scavenging enzymes consisting of catalase (CAT) and ascorbate-glutathione (AsA-GSH) cycle-related enzymes comprising ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). In this study, reduced AsA levels in hot water treated fruit increased and were maintained at a higher level than the control during storage, while oxidized AsA levels in treated fruit were maintained at lower levels during storage. Reduced GSH levels in hot water treated fruit increased and were maintained at higher levels than the control during storage, while oxidized GSH levels in treated fruit were maintained at lower levels during storage. CAT activity in treated fruit was higher than in the control and tended to decline during storage. Treatment with hot water before cold storage increased the activities of APX, DHAR and MDHAR more than the control during storage. GR activity initially increased, then slightly declined but remained at higher levels than the control.

Red sweet pepper fruit treated with hot water at 45 °C for 1 min exhibited CI mitigation during storage at 5 °C. Hot water treatment enhanced CAT activity and the AsA-GSH cycle by increasing reduced AsA and reduced GSH levels, and inducing APX, DHAR, MDHAR and GR activities. Thus, hot water treatment at 45 °C for 1 min induced CI tolerance in red sweet pepper fruit by activating CAT enzymes and the AsA-GSH cycle due to increasing antioxidant levels and related enzyme activities.

Chapter 3: Gene expression related heat shock proteins and antioxidant enzymes to hot water treatment of red sweet pepper fruit during low temperature storage

Heat stress induces the production of a group of proteins called heat shock proteins (HSPs) as stress-responsive family proteins related with CI resistance. HSPs function as membrane stabilizers and ROS scavengers, with synergic action in the antioxidant system. Fruit treated with hot water at 45 °C for 1 min had higher expression of *HSP70*. *CaCAT*, *CaAPX1*, *CaAPX3*, *CaMDHAR* and *CaGR* genes were induced in hot water treated fruit. The gene expression levels of CAT, APX, MDHAR and GR showed similar trends to CAT, APX, MDHAR and GR activities.

The observed improvement in the protection of red sweet pepper fruit against low temperature stress by hot water treatment at 45 °C for 1 min was explained by induction of the HSP70 gene, *CaCAT*, and AsA-GSH related enzyme genes including *CaAPX1*, *CaAPX3*, *CaMDHAR* and *CaGR*.

HSP70 gene expression was related to increased gene expression and activity of antioxidant enzymes. This induced synergistic action between HSPs and the antioxidant systems, resulting in improved membrane integrity and CI tolerance.

In conclusion, hot water treatment at 45 °C for 1 min mitigated CI and decay, and maintained quality such as color, firmness and total AsA level. Hot water treatment mitigated CI during low temperature storage by inducing the AsA-GSH cycle and enhancing antioxidant levels and antioxidant system-related enzyme activities. CI mitigation was related to increased gene expression of HSP70, antioxidant enzyme genes such as *CaCAT* and ASA-GSH cycle-related genes, consequently resulting in lower oxidative damage and lipid peroxidation. Results indicated that hot water treatment improved the quality and antioxidant system of red sweet pepper fruit during low temperature storage as a suitable postharvest treatment.

審査結果の要旨

赤ピーマン（*Capsicum annuum* L.）果実は、ビタミンＡやビタミンＣの供給源として高い栄養価を有し、フェノール物質やカロテノイドの生理活性物質を含み、世界的に経済上重要な園芸作物として熱帯および亜熱帯地域で栽培されている。赤ピーマン果実は収穫後の日持ち期間が短い生鮮食品であるため、適切に貯蔵して品質を保持することが求められる。低温貯蔵は園芸作物の品質を保持し、日持ち期間を延長する有効な貯蔵方法である。しかし、赤ピーマン果実は低温感受性が高く、低温貯蔵中に低温ストレスによって果実の腐敗、水分損失および果皮のピッティングといった低温障害（CI）が発生し、品質の低下を招き、商品性を損ねることになる。

園芸作物の CI を緩和する方法として、高温処理、CA 貯蔵および MA 貯蔵などの物理的方法や植物ホルモンを用いる化学的方法などが研究開発されている。高温処理は安全で環境に優しく、効果的な方法である。高温処理には温湯、温風、蒸気が用いられているが、温湯は効率的な熱媒体であり、CI の緩和に有効な効果が期待される。しかし、低温貯蔵中での赤ピーマン果実の低温に対する反応や温湯処理による CI の緩和効果についてはまだ不明な点も多い。本研究では、低温貯蔵での温湯処理による赤ピーマン果実の品質保持および CI の緩和効果を明らかにして、温湯処理による CI 緩和のメカニズムを解明することを目的とした。

第 1 章では、温湯温度と処理時間の違いが低温貯蔵中の赤ピーマン果実の品質に及ぼす影響について検討し、最適な温湯処理条件を明らかにしている。処理温度と処理時間は、温湯処理の重要な条件であり、低温での果実の生理的反応は、温湯処理の温湯温度と処理時間により変化する。赤ピーマン果実を 55°C の温湯で 1、5、10 分間、45°C の温湯で 1、3、5 分間それぞれ処理した後、10°C、4 週間貯蔵した。55°C の温湯はすべての処理時間で、45°C の温湯では 10 分間の処理で果実に処理直後高温障害

が発生した。45 および 55°C とも 1 分間の処理では、貯蔵中腐敗は発生しなかった。また、貯蔵中の重量減少も少なかった。果皮色は温湯処理による影響がなく、貯蔵中も有意な差はなかった。45 および 55°C の 1 分間処理では、他の温湯処理と比べて、貯蔵中での CI の進行が緩やかであり、CI 発生の程度も小さく、CI の発生に関係する細胞からのイオン漏出量や脂質過酸化の指標となるマロンジアルデヒド（MDA）含量が低く保たれた。総アスコルビン酸含量は、いずれの処理も貯蔵中に徐々に減少する傾向であったが、45 および 55°C の 1 分間処理では他の温湯処理と比べて、高いレベルで低温貯蔵中推移した。これらのことから、45°C、1 分間の温湯処理が低温貯蔵中での赤ピーマン果実の品質保持および CI の緩和に効果的であることが明らかとなった。

第 2 章では、温湯処理が赤ピーマン果実の低温貯蔵中での抗酸化機構に及ぼす影響を、45°C、1 分間で温湯処理した赤ピーマン果実を 5°C、4 週間貯蔵して検討している。温湯処理は低温貯蔵中の CI 発生を軽減し、その進行を抑え、活性酸素種の過酸化水素（H_2O_2）および MDA 含量を低下させた。そのことは、温湯処理が H_2O_2 消去作用を活性化し、低温ストレスによる脂質過酸化を抑制することを示した。また、温湯処理は低温貯蔵中の還元型アスコルビン酸および還元型グルタチオンを高いレベルに保持し、酸化型アスコルビン酸および酸化型グルタチオンを低レベルに維持して、抗酸化酵素であるカタラーゼおよびアスコルビン酸－グルタチオンサイクルのアスコルビン酸ペルオキシダーゼ、モノデヒドロアスコルビン酸還元酵素、デヒドロアスコルビン酸還元酵素およびグルタチオン還元酵素の酵素活性を高めた。これらのことから、45°C、1 分間の温湯処理は、赤ピーマン果実の抗酸化機構を活性化させ、H_2O_2 を消去することで、低温貯蔵中の CI を緩和することが示された。

第 3 章では、温湯処理による低温貯蔵中での赤ピーマン果実の熱ショックタンパク質（HSP）および抗酸化酵素の遺伝子発現に及ぼす影響を 45°C、1 分間の温湯処理した赤ピーマン果実を 5°C、4 週間貯蔵して検討している。温湯処理は HSP70 の遺伝子発現量を高めるとともに、抗酸化酵素の遺伝子発現量を増加させた。これらの傾向は、低温貯蔵中での抗酸化酵素の活性変化と同様であり、HSP と抗酸化機構との相乗作用が、CI の緩和に寄与することが示された。

本研究は、45°C、1 分間の温湯処理が、赤ピーマン果実の低温貯蔵中での CI 発生を軽減し、品質保持に効果があることを示した。また、CI の緩和に温湯処理による抗酸化機構の活性化が、関係していることを明らかにし、HSP と抗酸化機構との相乗作用が、CI の緩和に寄与していることを示したことが評価される。本研究成果は園芸科学、食品科学への応用発展に大きく貢献するものである。よって、本論文の審査ならびに最終試験の結果と併せて、博士（応用生命科学）の学位を授与することを適当と認める。