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Physicochemical Quality Changes in Chinese Cabbage with Storage Period and Temperature: A Review

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Abstract

Background: Recent inquiries into high-quality foods have discussed the importance of the functional aspects of foods, in addition to traditional quality indicators such as color, firmness, weight, trimming loss, respiration rate, texture, and soluble solid content. Recently, functional Chinese cabbage, which makes up a large portion of the vegetables consumed in Korea, has been identified as an anticancer treatment. However, the investigation of practical issues, such as the effects of storage conditions on quality indicators (including functional compounds), is still limited. **Purpose:** We reviewed various studies on variations in the quality indicators and functional compounds of Chinese cabbage in response to different storage environments, focusing on storage temperature and storage period. In particular, we emphasized the effect of storage temperature and storage period on glucosinolate (GSL) levels, in order to provide guidelines for optimizing storage environments to maximize GSLs. Additionally, we used response surface methodology to propose experimental designs for future studies exploring the optimal storage conditions for enhancing GSL contents. **Review:** Large variations in quality indicators were observed depending on the cultivar, the type of storage, the storage conditions, and the harvest time. In particular, GSL content varied with storage conditions, indicating that either low temperatures or adequate air composition by controlled atmospheric storage may preserve GSL levels, as well as prolonging shelf life. Even though genetic and biochemical approaches are preferred for developing functional Chinese cabbage, it is important to establish a practical method for preserving quality for marketability; a prospective study into optimal storage conditions for preserving functional compounds (which can be applied in farms), is required. This may be achievable with the comprehensive meta-analysis of currently published data introduced in this review, or by conducting newly designed experiments investigating the relationship between storage conditions and the levels of functional compounds.

Keywords: Chinese cabbage, Functional compounds, Glucosinolate, Quality indicators, Storage conditions

Introduction

Cruciferous vegetables such as Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), cauliflower (*Brassica oleracea*), broccoli (*Brassica oleracea* var. *italica*), and mustard are widely consumed, but the importance of their functional aspects has only recently been emphasized (Fenwick and Heaney, 1983; Akpolat and Barringer, 2015). Brassicaceae are increasingly consumed as salads or sauerkraut; in Korea, the annual consumption of Chinese cabbage is over 50 kg per person, the highest amount among Brassicaceae (Lee et al., 2015).

Chinese cabbage is generally harvested between the

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summer and winter seasons. Chinese cabbage harvested in the late fall is typically stored, and is shipped 3-4 months later. Chinese cabbage harvested during the summer, however, is difficult to store for longer than a month because of high temperatures and wet weather, both of which cause quality degradation and weight loss (Bae et al., 2015; Kim et al., 2001). Therefore, maintaining appropriate air conditions, temperature, and humidity during storage is essential for preventing quality degradation (Kim et al., 2010; Eum et al., 2013a; Kim et al., 1998; Lee et al., 1994; Han et al., 1998).

Physicochemical indices such as color, firmness, sugar content, weight, and flavor have been used to evaluate the quality of Chinese cabbage. Studies have been conducted regarding the effects of storage temperature on weight variation and trimming loss (Klieber et al., 2002; Eum et al., 2013b). Researchers have also examined how changes in pH, storage time, temperature, and concentration of ascorbic acid affect the color of red cabbage (Tomczak and Czapski, 2007; Devahastin and Niamnuy, 2010). Recently, the consumption of brassica vegetables containing abundant functional components has increased, because the anticarcinogenic properties of these vegetables' carotenoids and glucosinolates (GSLs) have been emphasized (Jahangir et al., 2009; Akpolat and Barringer, 2015). Isothocyanate is a typical functional component of cruciferous vegetables, which is beneficial in the treatment of lung, bladder, and rectal cancers, and which has antibacterial and pesticidal properties (Morimitsu, 2000; Soledade et al., 1998; Cho et al., 2010; Cartea et al., 2008). Flavonoids are known to have anticancer effects and prevent heart disease (Nabasree and Bratati, 2007), while GSLs are well-known anticarcinogens (Traka and Mithen, 2009; Lee et al., 2015). GSLs in particular are functional components unique to Brassicaceae crops (Cartea and Velasco, 2008; Rodrigues and Rosa, 1999). The amount of GSLs varies throughout the plant-it is highest in the heart, followed by the stem and then the leaf (Shim et al., 1992). A role has been proposed for GSLs in cancer prevention, possibly resulting from the production of breakdown products such as isothiocyanate after myrosinase enzyme activity, both of which are beneficial to human health (Kim et al., 2010). Another study demonstrated that the types and amounts of GSLs in Chinese cabbage vary with genetic characteristics, growth phase, and climate (Agerbirk et al., 2001; Stoewsand, 1995). In addition, recent studies have examined the effects of culinary processing and cooking methods on GSL amounts (Song and Thornalley, 2007).

Although the GSL content of cruciferous vegetables varies according to various factors such as plant organ, cultivation period, and cultivation conditions (Bérard and Chong, 1984; Rungapamestry et al., 2006), post-harvest processing (e.g., storage) also significantly affects GSLs (Rosa et al., 1996; Kushad et al., 1999; Verkerk et al., 2001). Lee et al. (2015) reported that the total GSL levels in Chinese cabbage gradually declined when the Chinese cabbage was stored at ; another study, however, showed that the total GSL content of broccoli increased by 42% over seven days of storage under an air composition of 0.5%O_2+20%CO_2 (Hansen et al., 1995).

Because the active trade of agricultural products currently results in lengthy distribution durations, appropriate storage conditions and techniques for maintaining the quality of agricultural and food products are required. However, most studies of GSLs have focused on genetic, biochemical, and breeding issues, rather than on storage environments for optimizing functional components (including GSLs). This study reviews changes in quality indices (including functional components), during the storage of Chinese cabbage. This paper begins with an introductory review of how changes in physicochemical quality indices and a few notable functional components are related to storage conditions. The paper then proceeds to a specific review focusing on how GSL changes depend on temperature and storage periods. This review provides valuable information for prospective studies on optimizing the production of functional components in cruciferous vegetables, including Chinese cabbage. Finally, this study proposes several experimental designs for investigating and optimizing GSL contents in response to storage environments. These designs use response surface methods (RSM) and describe the optimal number of measurements (trials) and levels of storage conditions for such experiments.

Review of quality variation in Chinese cabbage depending on temperature and storage period

Inappropriate handling during postharvest processing operations (including harvest, transportation, and storage) can damage vegetables and fruits. Chinese cabbage is easily damaged by physical impact, which causes subsequent volume and weight loss (Han et al., 2000; Kim et al., 2009; Jeong et al., 2011). Productivity and quality vary heavily depending on the harvest season and storage period (Bae et al., 2015); this variability leads to significant temporal fluctuations in price, demand, and supply (Han et al., 2000; Kim et al., 2009; Jeong et al., 2011). Additionally, the commercial value of Chinese cabbage decreases rapidly as the quality of the cabbage deteriorates-the cabbage spoils quickly and has a short shelf life (Eum et al., 2013a; Kang et al., 1999). Koh et al. (1993) reported that the maximum time Chinese cabbage could be stored without quality deterioration was about three months; storage beyond this period led to decreases in quality.

Harvested Chinese cabbage can be classified into spring cabbage, summer cabbage, autumn cabbage, and winter cabbage (Kim et al., 2007; Kim et al., 2010). Each variety contains different amounts of functional components and exhibits unique characteristics in terms of production, harvest, and post-harvest processes. In particular, storage conditions are crucial for maintaining the quality of Chinese cabbage, including the amount of functional components. Hence, it may be necessary to create a database identifying the quality of cabbage stored under various conditions, to aid in establishing guidelines for maintaining high quality. However, no existing studies have comprehensively reviewed the effects of storage conditions. Here, we review the literature on changes in Chinese cabbage's physicochemical qualities and levels of functional components (with a particular focus on GSL) during storage, as a basis for building such a database.

Physical and physicochemical quality of Chinese cabbage

Traditionally, various indicators have been used for determining the quality of Chinese cabbage, including color, firmness, weight, trimming loss, respiration rate, texture, and soluble solid content (see Table 1). Color is a simple quality index, as it can be easily evaluated with the naked eye (Ren et al., 2006). Color changes have been reported in red cabbage stored at 10°C, 20°C, and 30°C (to a greater extent at 30°C than 10°C) with decreased brightness and visibility; stable color was observed at (Tomczak and Czapski, 2007). Another study showed that storage in a controlled atmosphere (CA) ($\%CO_2/O_2\%=1/1$) for two months was best for preventing chromaticity changes in Chinese cabbage (*Brassica campestris* L. cv. Gonaengjee-yureum) (Yang et al., 1993a). Firmness is exhibited differently in different Chinese cabbage varieties, the general trend being that the inner leaf is firmer, whereas mid-rib firmness decreases within the same head (Jeong et al., 2012). Another study on the Chinese cabbage cultivars 'CR-Nongshim' and 'Ryouckgwang' reported that storage temperature did not significantly affect firmness (Eum et al., 2013b). Weight is the quality indicator that deteriorates the most during storage. In general, weight loss is expressed as a percentage ratio of the weight loss during storage to the initial weight. Based on the results of previous studies, up to 20% of weight loss may be dependent on storage temperature and cultivar variety, with storage conditions at low temperatures (2°C) and net packing of 'Choongwang' Chinese cabbage harvested in the summer (Eum et al., 2013a). Additional studies have investigated the effects of a wider range temperatures (0°C, 2°C, 5°C and 25°C at 90% relative humidity (RH)) on weight loss in Chinese cabbage ('CR-Nongshim' and 'Ryouckgwang' cultivars) (Eum et al., 2013b). 'CR-Nongshim' and 'Ryouckgwang' stored at showed weight loss of more than 15%, the two cultivars showed losses of over 18% and 20% at , respectively. However, the weight loss of 'CR-Nongshim' and 'Ryouckgwang' cultivars stored at 2°C was approximately 10%. This study did not demonstrate a clear correlation between weight loss and storage temperature, as weight loss fluctuated with temperature, indicating that further study is necessary. In contrast to typical storage, Yang et al. (1993a) demonstrated that controlled atmospheres ($\%CO_2/O_2\% = 1/1, 5/3, \text{and } 7/15$) may prevent significant weight loss during storage.

Trimming loss is usually represented by the percentage change toward pre-removal weight after removing a damaged outer leaf (Lee et al., 2007). The previously mentioned study by Eum et al. (2013b) also illustrated that trimming loss varied between cultivars, storage periods, and storage temperatures. That study showed that the trimming loss for 'CR-Nongshim' was initially highest for cabbages stored at 0°C, followed by trimming loss for cabbages stored at 5°C and 2°C; however, no significant difference was observed after three weeks of storage. In contrast, 'Ryouckgwang' had the lowest trimming loss at 5°C after two weeks, but the highest trimming loss at 5°C

Table 1. Notable studies that have investigated changes in the quality of cabbage during storage

Reference	Cultivar	Storage conditions	Storage period	Quality Indicators	Result
Zhang et al. (2013)	Brassica rapa L. Chinensis Group	2°C~15°C	0~26 days	Color Respiration Weight	All quality factors change more slowly because of reduced physiological activities of Chinese cabbage & extended shelf-life period at low temperature (2°C)
Kramchote et al. (2012)	Brassica oleraceae var. capitata L.	4°C~28°C (95±1% RH)	12~18 days	Color Weight Firmness Respiration TSS*	All quality factors were maintained effectively during storage at low temperature (4°C)
Eum et al. (2013b)	Ryouckgwang & CR-nongshim	0°C~25°C (90% ↑ RH)	0~30 days	Weight Trimming loss Firmness SSC Sensory scores	The optimal storage temperature for maintaining quality for both cultivars was 2°C
Tomczak & Czapski (2007)	Red cabbage 20 WSP	10°C~30°C	0~30 days	Color	Generally, color was found to drop with increases in storage time. Temperature and color were stable at lower temperatures
Yang et al. (1993a)	Brassica campestris L. cv. Gonaengjee-yureum	(%CO_2/%O_2= 1/1, 5/3, 7/15)	2 months	Color Firmness Weight loss	There was no significant difference in weight loss. Color and firmness were effectively maintained under (%CO_2/%O_2=1/1) conditions

*TSS: Total soluble solids, SSC: Soluble solids contents

after four weeks. Another study investigated the effects of storage temperature and storage period on trimming loss in the Chinese cabbage cultivar 'Yuki', with 47% loss recorded at 20°C after one week. Trimming loss was lower at decreased storage temperatures (Porter et al., 2003). At a storage temperature of 2°C, trimming loss was only 44%, even after nine weeks. Conversely, Klieber et al. (2002) reported that trimming loss did not depend on harvest time or storage period. Finally, trimming loss can improve marketability by removing the damaged outer leaves of Chinese cabbage (Eum et al., 2013b).

Basic physiological activities should also be considered, such as respiration rate before and after harvest (Wu, 2010). Several studies have suggested that respiration rate can be used as a measure of quality, which affects shelf life and depends on storage temperature (Bruzo, 1980; Yang et al., 1993b). When Chinese cabbage (*Brassica rapa* L. Chinese Group) was stored at 2°C and 6°C, its respiration rate was shown to decrease up until six days of storage, after which the rate consistently increased. Further, the respiration rate rapidly increased at 10°C and 15°C during short storage periods (Zhang et al., 2013). Another study also suggested that low storage temperature resulted in lower respiration rates, with the rate for Chinese cabbage stored at 28°C being 2-3 times higher than cabbage stored at 4-10°C (Kramchote et al., 2012). It has also been reported that Chinese cabbage stored at 20°C has a higher respiration rate than cabbage stored at low temperatures of 0°C and 2°C. A drastic increase in respiration rate was related to senescence (Porter et al., 2003).

Texture is an important quality indicator because it changes significantly depending on the length of storage period. A study on the effect of post-harvest processing on Chinese cabbage texture during storage at 4°C illustrated that Chinese cabbage processed with reddish soil did not show any texture changes compared to the control group, while Chinese cabbage processed with natural red clay had a better texture after a short storage time (Seo et al., 2015). Another study investigated the elasticity of Chinese cabbage that had been salted after storing at 0-2°C for 0-2 weeks. Elasticity was reduced by 50% after four weeks (Jeong et al., 2011). A few studies have investigated changes in soluble solid contents depending on the cultivar (Lee et al., 2013b), methods of packaging and salting (Lee et al., 2007), and preprocessing treatment (Bae et al., 2015). Lee et al. (2013b) investigated how changes in soluble solid contents were related to the type of cultivar; they showed that the soluble solid contents were the highest in 'Chunkwang' and the lowest in 'Jeongsang'. The effect on the quality of the 'Choongwang' cultivar of packaging with polyethylene (PE) film sacks, plastic containers, and polypropylene (PP) was also studied (Lee et al., 2007). That study showed that soluble solid contents were not affected by the type of packaging, but physical quality indicators (such as weight loss rate and appearance) were retained better in cabbages packaged with PE film sacks. In addition, Bae et al. (2015) examined the effects of pre-processing (forced air cooling, room cooling, and pre-drying) on changes in soluble solid contents in 'Choongwang' during storage. However, studies on storage temperature-dependent changes in the texture of Chinese cabbage were insufficient to draw conclusions.

Appearance is another quality measure that can be assessed by sensory tests (Kays, 1997; Eum et al., 2013a; Bae et al., 2015). Generally, the appearance of Chinese cabbage is evaluated on a nine- or ten-point scale (Bae et al., 2015). When the 'Choongwang' cultivar was stored at 2°C for six weeks, its sensory score for appearance was reduced from 6.6 to 4.4, indicating that longer storage negatively affects appearance (Eum et al., 2013a). However, Lee et al. (2013a) showed that lower storage temperature may prevent drastic changes in appearance. They showed that a winter-grown cabbage (*Brassica campestris* L.) stored at -20°C for 12 months did not decrease significantly in appearance, as measured by sensory testing.

In summary, quality is generally maintained at low storage temperatures. Marketability could be further increased by identifying optimal storage conditions, such as optimal atmospheric compositions for controlled atmosphere (CA) storage.

Changes in levels of functional components of Chinese cabbage

Beneficial functional components in Chinese cabbage include:chlorophyll, which is an antioxidant that combats cancer and reduces cholesterol (Lanfer-Marquez et al., 2005; Seong et al., 2006); ascorbic acid, which is involved in maintaining food quality, and is an antioxidant that prevents the peroxidation of fat (Hu et al., 2007); polyamine, which acts in lignin resynthesis, the protection of biological membranes, and the regulation of osmotic pressure (Bouchereau et al., 1999; Marton and Morris, 1987; Hwang et al., 2004); GSL and its derivatives (Mithen et al., 2000); phenol (Zhang et al., 2013); and amino acids (Hong and Kim, 2006) (Table2).

Chlorophyll content is an indicator not only of quality, but also of the levels of useful functional components. One study compared Chinese cabbages treated with dimethyl dicarbonate (DMDC) during the first four days of storage to those treated with a sterile water dip (control group), stored at 4°C for eight days (Chen et al., 2013; Jacxsens et al., 2002). They showed that, for the first four days of storage, the chlorophyll content of DMDC-treated Chinese cabbage was considerably lower than that of the cabbage the control group. However, the contents became similar for the last four days of storage in the the DMDC-treated sample. Zhang et al. (2013) investigated how varying temperatures and storage periods affected chlorophyll content, and observed that that content was reduced for all tested storage temperatures. The decline of chlorophyll may be due to decreasing magnesium content during storage (Martins et al., 2005). Another study observed that the chlorophyll content of cabbage stored at for 12 days decreased by 65%, while the chlorophyll content of cabbage stored at for 18 d decreased by 4.2% and 39.6%, respectively (Kramchote et al., 2012). Chlorophyll a and b content was reduced at 1°C when cabbage was stored for two months (Yang, 1994). Based on this previous evidence, it is conclusive that chlorophyll content is inversely proportional to storage temperature.

Ascorbic acid has been reported to have direct anti-aging and anticancer effects (Park, 1995). According to a previous study, the levels of ascorbic acid in Chinese cabbage stored at 2°C decreased more slowly than those of cabbage stored at 6°C, 10°C, or 15°C. Another study also suggested that low storage temperatures had beneficial effects on ascorbic acid levels through comparison of cabbage stored at 4°C, 10°C, and 28°C (Kramchote et al., 2012):Chinese cabbage stored at 2°C maintained the highest levels of ascorbic acid. This may have been due to inhibited enzymatic activity (Zhang et al., 2013), and, consequently,

Table 2. Studies on variation in functional compound contents in cabbages depending on storage conditions

Reference	Cultivar	Storage conditions	Storage period	Functional compounds	Result
Zhang et al. (2013)	Brassica rapa L. Chinensis Group	2°C~15°C	0~26 days	phenol ascorbic acid chlorophyll	Low temperatures (2°C and 6°C) are recommended to maintain functional compounds during the preservation of Chinese cabbage
Kramchote et al. (2012)	Brassica oleraceae var. capitata L.	4°C~28°C (95±1% RH)	12~18 days	ethylene chlorophyll ascorbic acid	All functional compounds decreased less during low-temperature (4°C) storage
Hong & Kim (2006)	Winter pride & 55days (Brassica campestris L. Perkinensis)	4°C	1~4 months	vitamin U amino acid methionine	Vitamin U contents were increased at 4°C and methionine was not detected during storage
Hwang et al. (2012)	Brassica campestris L. ssp. pekinensis	4°C	7 days	polyphenol flavonoids	Storage at a low temperature (4°C) reduced losses in amounts of functional compounds; 4°C might be the optimal temperature for preserving these compounds
Wang (1988)	cv. Kyoryoku 60	0°C in air 0°C in 1% O_2	0~12 weeks	polyamine	Polyamine declined more slowly in 1% 0_2 than in air
Yang et al. (1993a)	Brassica campestris L. cv. Gonaengjee-yureum	(%CO_2/%O_2= 1/1, 5/3, 7/15)	2 months	chlorophyll	Chlorophyll contents were reduced by 18.5% at 1/1, 37.5% at 5/3, suggesting 1/1 condition was optimal for preserving chlorophyll

limited degradation at low storage temperatures (Hirata et al., 1987). Generally, the degradation of ascorbic acid depends on temperature, harvest season, and processing method (Fennema, 2007).

Polyamine protects cell membranes from cell lysis (Mager, 1959), is involved in tRNA (Cohen et al., 1969), and stabilizes both DNA and tRNA molecules (Bachrach, 1973; Cohen, 1971). One study investigated the effects of atmospheric composition during storage on polyamine content (Wang, 1988). This research showed that polyamine levels, sampled from many parts of a Chinese cabbage (inner/outer laminae and midribs), decreased after six weeks of storage at 0°C in air; however, high polyamine levels were sustained in a 1% O_2 atmosphere.

Isothiocyanate, a breakdown product of GSL, is a component of a defense system that protects plants from attack by insects, bacteria, and microorganisms (Chew, 1988). Owing to its favorable anticarcinogenic effects, it has recently come under the spotlight as a functional component that improves human health (Fahey et al., 1997; Barrett et al., 1998). Additionally, allyl isothiocyanate is obtainable by conversion of sinigrin by myrosinase (Yen and Wei, 1993). A previous study conducted by Akpolat and Barringer. (2015) indicated that the allyl isothiocyanate content in green cabbage (*B. olerace*a var. capitata) stored at 4°C and 25°C decreased steadily with longer storage time, although it was not significantly affected by storage temperature. Another study compared volatile isothiocyanate production between stored 'Safekeeper cabbage' and 'F1 Mercury cabbage' (*B. olerace*a L. var capitata L.) stored at 1±1 (Bérard and Chong, 1984). The conclusion of this study was that the amount of volatile isothiocyanate fluctuated in 'Safekeeper cabbage', while it slowly increased in 'F1 Mercury cabbage', highlighting the differences between cultivars.

Phenolic acids are secondary metabolites that have physiological and medical properties involved in secreting bile in animals and reducing sap in plants (Tattini et al., 2004; Ghasemzadeh and Ghasemzadeh, 2011). Increases in phenolic acid levels could protect plant quality from the first day after harvest depending on environmental conditions (Starzyńska et al., 2003). When storing white cabbage (*B. oleraceae* L. var *capitata* L. *alba* DC. Cv Bison) at 1°C, the levels of seven out of 11 phenolic acids were maintained in the plant during the storage period, while two phenolic acids were lost (Hounsome et al., 2009). Another study showed that the total phenol content of Chinese cabbage stored at increased until 15 days of storage period, and then decreased, suggesting that long-term storage could cause a decrease in phenol content (Zhang et al., 2013).

Vitamin U (S-methylmethionine) is a naturally synthesized bioactive substance (Gessler et al., 1991b; Maw, 1981). It is known as an antiulcer factor, and its deficiency causes stomach ulcers. In addition, it is anti-inflammatory, a painkiller, and is radioprotective (Leung and Leung, 1989; Gessler et al., 1991a). A study demonstrated that vitamin U content depended on cultivar (Hong and Kim, 2006). Researchers stored 'Winter cabbage' and '55 days cabbage' at 4°C for one week, after which increased vitamin U and methionine (a precursor of vitamin U) levels were observed.

Several other studies have examined anthocyanin, which is a flavonoid (Jack, 1998; He and Giusti, 2010). Tomczak and Czapski (2007) investigated changes in anthocyanin concentrations in red cabbage resulting from storage temperature, showing that anthocyanin levels gradually decreased with increasing temperature, from 10°C to 30°C over 30 days. A study regarding flavonoids in Chinese cabbage reported that 15 varieties of flavonoids were present, but only six types (chalcone, equol, flavone, hydroxyflavone, pelargonidin, and purpurogallin) were maintained during storage (Hounsome et al., 2009). The study also showed that two types of flavonoids (artemetin and cyaniding) degraded after three months of storage, with the rest disappearing after five months. Notably, they demonstrated that the levels of some flavonoids increased towards the end of the storage period, owing to plant responses to fungal infection. Hwang et al. (2012) measured the flavonoid content of Chinese cabbage stored at 4°C for 7 days, showing that it decreased overall, despite a slight increase following the first day of storage.

Changes in GSL levels in Chinese cabbage

GSLs are well-known functional components with anti-carcinogenic effects, and are unique to Cruciferae (Verkerk et al., 2001). In addition, the breakdown products of GSLs contribute to the unique flavor and fragrance of Brassicaceae vegetables (Van Doorn et al., 1998; Macleod, 1976), and also act in plant defense mechanisms due to their significant physiological activities (Grubb and Abel, 2006). For this reason, most recent studies have focused on the biochemical and genetic aspects of GSLs. Investigations into the influence of post-harvest conditions on GSLs have been limited (see Table 3).

In general, Chinese cabbage is distributed after storage at 4-8°C, and its nutrient content (including its GSL content) varies with storage period and temperature (Kelly et al., 1998; Lee et al., 2015). Shim et al. (1992) investigated changes in the GSL content of Chinese cabbage (*B. pekinensis* Rupr) packed into thin plastic bags and stored at 8°C ; they observed a decrease in GSL content from 6.1 mM/g to 5.0 mM/g after 20 days of storage. Another study reported that total GSL in cabbage [*B. oleracea* L. (Capitata group)] showed substantial fluctuations during storage in refrigerated air (5 to 215 days) (Chong and Berard, 1983). Besides Chinese cabbage, GSL content in pakchoi, which also belongs to the Cruciferae, was studied in storage at 4-20°C. The GSL content in pakchoi stored at 20°C for 9 days was about 47% lower than that in pakchoi stored at 4°C (Yang et al., 2010). In addition, the direction of change in GSL levels differed with temperature. GSL levels in pakchoi stored at 4 did not change significantly during the first three days of storage, increased over the next 5-7 days, and were reduced to original levels after nine days. In contrast, pakchoi stored at 20°C exhibited an initial increase in GSLs, which was then followed by a gradual decrease. This study suggests that the optimal storage period varies with storage temperature. Lee et al. (2015) reported that aliphatic GSLs and indolyl GSLs in Chinese cabbage (grown for 90 days) decreased 64% and 48-57%, respectively, when stored at 4, suggesting that aliphatic, indolyl, and aromatic GSLs might not be preserved at low temperatures. Another study reported that indolyl GSLs are more sensitive to storage conditions than aliphatic and aromatic GSLs (Verkerk et al., 1997). Moreover, 'Safekeeper cabbage' and 'F1 Mercury' cultivars were refrigerated in a controlled atmosphere (2.5% O_2, 5% CO_2), in order to investigate changes in total GSL content (Bérard and Chong, 1984). In that study, GSLs in the first cultivar decreased substantially for eight days, then increased gradually, while they fluctuated (with a slight increase) in the latter. A trend common to both cultivars was that GSLs initially showed a slight increase, and then

Table 3. Studies on changes in GSLs in cabbages depending on storage conditions and harvest season

Reference	Cultivar	Storage conditions	Storage period	GSL change[a]	Result
Shim et al. (1992)	Brassica pekinensis Rupr	8°C	20 d	6.2 to 5.0 mM/g[a1]	GSLs were reduced by storage.
Lee et al. (2015)	Brassica rapa L. ssp. pekinensis	4°C	72 h	13.1 to 7.41 μmol/g[a2]	GSLs were not preserved over long periods at a low storage temperature (4°C).
Bérard & Chong (1984)	Cabbages cvs safekeeper & F1 Mercury (Brassica oleracea L. var capitata L.)	1±1°C (95% RH)	1-214 d	907 to 1419 μg/g[a3] & 1526 to 1918 μg/g[a3]	GSLs increased slowly during the first stage, and showed rapid increases at the end of a storage period at a low temperature (1±1°C).
Hwang et al. (2012)	Brassica campestris L. ssp. pekinensis	4°C 21~23°C	7 d	13.55 to 13.18 μmol/g[a4] 13.55 to 10.04 μmol/g[a4]	The total amount of GSLs did not change significantly at a low storage temperature (4°C), but decreased at room temperature (21~23°C).
Chong & Bérard (1983)	F1 Mercury, Hidena, Safekeeper (Brassica oleracea L.(Capitata group))	1±1°C	5-215 d	F1 Mercury : 2019 μg/g[a3] Hidena : 1428 μg/g[a3] Safekeeper : 1280 μg/g[a3]	There were substantial fluctuations in GSLs and total GSLs yields over the storage period, ranked as F1 Mercury > Hidena > Safekeeper.

[a]total GSL contents ([a1]Sinigrin, Gluconapin, Progoitrin, Glucotropaeolin, Glucobrassicin, 4-Hydroxyglucobrassicin; [a2]Progoitrin, Sinigrin, Gluconapoleiferin, Gluconapin, 4-Hydroxyglucobrassicin, Glucocochlearin, Glucobrassicanapin, Glucobrassicin, 4-Methoxyglucobrassicin, Gluconasturtiin, Neoglucobrassicin; [a3]Volatile isothiocyanates, Thiocyanate ion, Goitrin; [a4]Progoitrin, Glucoalyssin, Gluconapin, Glucobrassicanapin, Glucobrassicin, 4-Methoxyglucobrassicin, Neoglucobrassicin)

tended to increase sizably toward the end of storage. Both a controlled atmosphere and refrigerated storage produced a similar pattern in GSL levels. Another study showed that GSL levels in brassica vegetables stored at 10°C were increased by 42% (Hansen et al., 1995). Hwang et al. (2012) reported that the total GSL content of Korean cabbage (*B. campestris* L. ssp. *pekinensis*) showed little change after seven days at 4°C, but notably decreased at ambient temperatures (21-23°C). Consequently, lower temperatures may be optimal for long-term storage, as there is a significant reduction in functional compounds (i.e., GSLs) at ambient temperatures. Other studies have investigated the effects of storage conditions on cauliflower (*B. oleracea* var. botrytis cv. Freemont). One such study investigated changes in GSL content in cauliflower heads harvested at different dates and stored at 0 in either air or a controlled atmosphere (3% O_2, 5% CO_2); that study showed that GSL content increased for 28 days and then stabilized. There was also no significant difference in total GSL level between vegetables stored in either a controlled atmosphere or air (Hodges et al., 2006). Another study investigated changes in aliphatic and indole GSL levels, specifically, after boiling cauliflower that had been stored frozen for a prolonged period (Volden et al., 2009). The GSL levels were analyzed according to harvest season and variety (Kim et al., 2010). Additional studies have investigated GSL levels in white cabbage (*B. oleracea* var. *capitata*) after treatment with heat and white vinegar containing acetic acid (Wennberg and Nyman, 2004). These studies have looked at GSL levels at different points in time after boiling (Volden et al., 2008), after processing depending on harvest season (Kim et al., 2010), and have examined the total GSL content of red cabbage after microwave processing (Verkerk and Dekker, 2004). In addition, one study observed fermentation-conditiondependent changes in the GSL content of white cabbages harvested by season (Villaluenga et al., 2009). Studies have evaluated changes in GSL levels for vegetables other than cabbage, including changes in GSLs of rocket salad (*Eruca sativa* Mill.), another cruciferous plant, depending on storage temperature and duration (Kim and Ishii, 2007), changes in GSLs in growing kale (*Brassica oleracea* acephala group) (Velasco et al., 2007), and changes in GSLs in broccoli during storage in a controlled

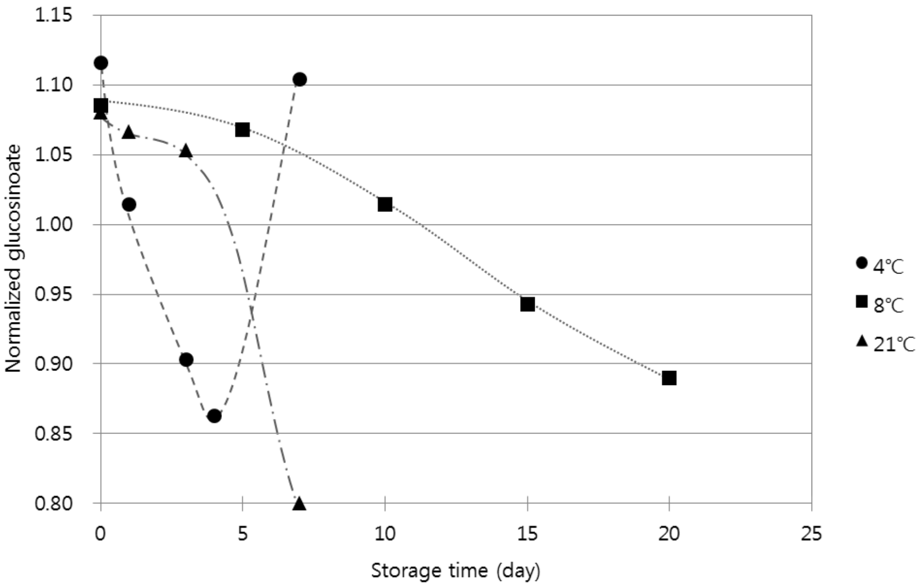


Figure 1. Simple comparison of trends in GSL reduction over storage time. The analysis was performed based on previously published data (Lee et al., 2015; Shim et al., 1992; Hwang et al., 2012), and shows that storage at room temperature (21°C) elicits a more significant decrease in GSL compared to other (lower) temperatures.

atmosphere for seven days (Hansen et al., 1995).

Conclusively, low storage temperatures (e.g., 0°C and 4°C) are effective for preserving GSL content, but deterioration over time is inevitable. In addition, a controlled atmosphere seems to be better for maintaining GSL content during long-term storage, as long as atmospheric conditions are optimized. It is important to note that the optimal temperature and amount of GSL vary between cultivars, and that these depend on post-harvest processing, suggesting that optimal storage conditions must be determined for each cultivar. In the future, a study that aims to preserve the functional components of Chinese cabbage by optimizing environmental storage parameters such as air composition, humidity, and temperature is required. This will enable the distribution of cabbage of maximum quality.

Experimental design Previous data analysis

We used previously published experimental data (Lee et al., 2015; Shim et al., 1992; Hwang et al., 2012) to analyze the effects of storage temperature (at 4°C, 8°C, and 21°C) on GSL content, and to propose possible future studies. To minimize the variation caused by different experimental designs, we normalized the collected data. However, because normalization cannot explain all variations between the studies, we focused on trends of GSL reduction and normalized reduction ratio at different temperatures, calculated as (initial amount- minimum amount, divided by initial amount). The analysis showed that the trend of reductions to GSL levels differs significantly depending on storage temperature (figure 1). At 4°C, which is suggested as the optimal storage temperature, there were notable fluctuations. GSL content of cabbage stored at 8°C and 21°C decreased with storage time. In addition, reduction rates were slower at 8°C than 21°C, consistent with the conclusion that low temperatures are better for Chinese cabbage. This assessment is also confirmed by the reduction ratios, which were 0.03, 0.18 and 0.26 at 4°C, 8°C, and 21°C, respectively, meaning that GSL content was reduced more for cabbage stored at 8°C and 21°C than at 4°C. However, it should be noted that this analysis only comprised a small number of data points. Unfortunately, no study investigates changes in GSL levels depending on storage humidity. Because agricultural products are stored at humidity levels of over 90%, but individual farmers use different storage temperatures, it is necessary to conduct an investigation into the effects of storage temperature on GSL content. Of course, if both humidity and temperature during storage can be considered, it would be better to optimize the storage conditions to select a reasonable shelf life for Chinese cabbage in terms of its functional components. For example, the RSM (combined with its corresponding experimental design, e.g., central composite design, simplex design, equiradial design, etc.) will calculate the optimal conditions for storing Chinese cabbage with minimal experimental cost and conditions.

RSM experimental design for studying the dependence of GSL content on storage conditions

RSM is a statistical method that optimizes a response variable (dependent variable) according to multiple explanatory variables (independent variables) (Neter et al., 1996). RSM visualizes the response of the target depending on varying experimental conditions, using a regression equation containing an intercept, and coefficients of first-order, secondorder, and interactive terms (Eq. (1)).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{kk} X_k^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \qquad (1)$$

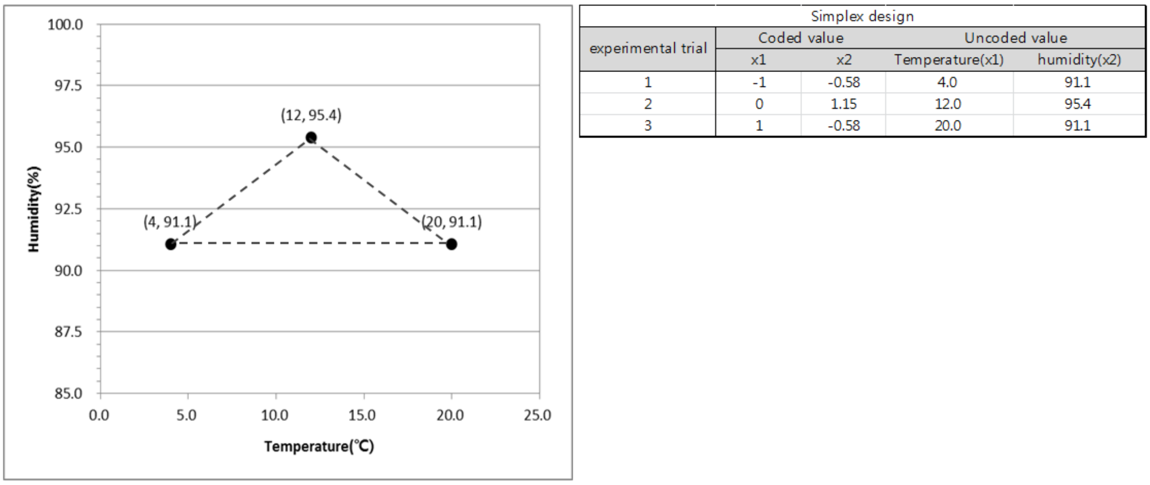
$X_{i,j,k}$ is the ith, jth, and kth coded value converted by Eq. (2) β_i is the regression coefficient.

$$X_j = \frac{Actual\ Level - \dfrac{High\ Level + Low\ Level}{2}}{\dfrac{High\ Level - Low\ Level}{2}} \qquad (2)$$

Response surface experimental design is a method for designing RSM experiments (Montgomery, 2008) that aims to identify the optimal experimental trial number (number of data points) and number of conditions (range of experimental variables) according to the order of the regression model. Generally, the number of trials in a experiment is composed of central points, factorial points, axial (or star) points, which vary depending on the number of variables. In this study, we identified three widely used response surface experimental designs applicable for future study into the effects of storage conditions (temperature and humidity) on GSL content:simplex, central composite, and equiradial.

Simplex design

Simplex designs are used for fitting first-order models by designing regularly sided patterns. This type of design has k+1 corners for k independent variables in k dimensions (Spendley et al., 1962). For instance, we can establish a three-trial experimental design with two and three levels of coded parameters for two variables. Herein, we attempted to design an experiment with three levels of storage temperature and two levels of humidity. The minimum and maximum storage temperatures were 4°C and 20°C, respectively, while humidity points were automatically determined in a given range of 90~95%. Figure 2 illustrates the results for response surface experimental design under the above conditions, showing a triangle-pattern design composed of storage temperatures of 4°C, 12°C, and 20°C, and humidity levels of 91% and 95%.

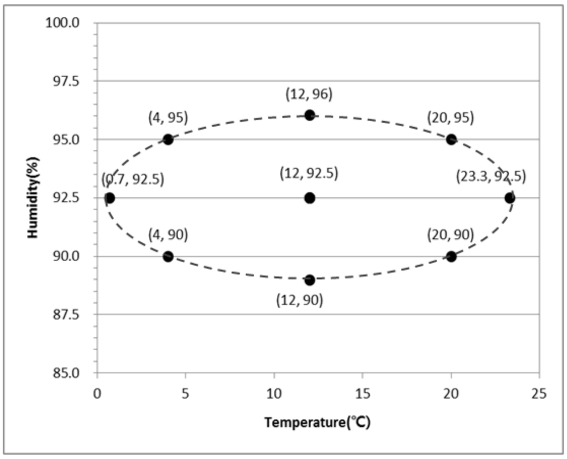


Simplex design				
experimental trial	Coded value		Uncoded value	
	x1	x2	Temperature(x1)	humidity(x2)
1	-1	-0.58	4.0	91.1
2	0	1.15	12.0	95.4
3	1	-0.58	20.0	91.1

Figure 2. Response surface experimental design using the simplex design method. This design is composed of three levels of temperature and humidity for Chinese cabbage storage, and is useful when experimental resources are limited.

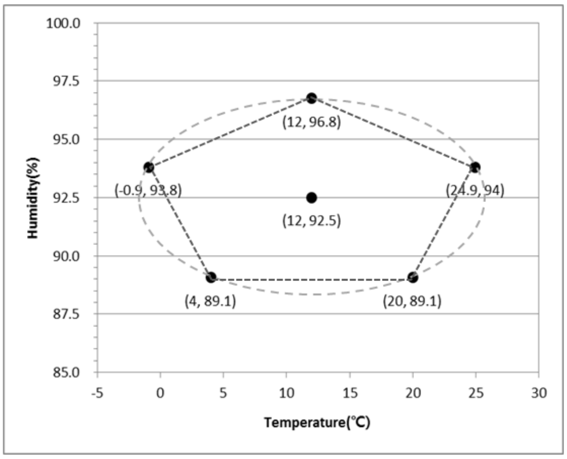
Central composite design

Central composite design (CCD) is the most popular design in RSM, and is useful for designing second-order models. The experimental trial is composed of 2^k factorial points, 2k axial (or star) points, and n central points, where factorial and axial points are necessary for determining the parameters of a quadratic model (Montgomery, 2008). In order to develop a highly accurate RSM model it is also important to select the number of repeats for central points and the distance (α) between central points and axial points. In general, 3-4 repeats are recommended for the central points. The importance of α is characterized by the rotatability. For better predictability in second-order models, constant and stable variance is required, which can be achieved when the measurement spot is distributed at the same distance (that is, a rotatable circular or spherical form). Thus, the locations of axial points are determined by the cross points of a circumscribed circle of a figure that is formed by factorial points. For example, when two independent variables are used, we can design an experiment with 11 trials, with five levels for each independent variable, resulting from 2^2 factorial points, 2×2 axial points with $a = \sqrt{2}$ and three central points. By applying this experimental design to an investigation of the effect of storage temperature and humidity on GSL levels in Chinese cabbage, we can set up the experimental design shown in figure 3. In this design, the standard storage temperatures were 4°C and 20°C, with five levels, and humidity ranging from 89% to 96%.



Central composite design				
experimental trial	Coded value		Uncoded value	
	x1	x2	Temperature(x1)	humidity(x2)
1	-1	-1	4.0	90.0
2	1	-1	20.0	90.0
3	-1	1	4.0	95.0
4	1	1	20.0	95.0
5	-1.41	0	0.7	92.5
6	1.41	0	23.3	92.5
7	0	-1.41	12.0	89.0
8	0	1.41	12.0	96.0
9	0	0	12.0	92.5
10	0	0	12.0	92.5
11	0	0	12.0	92.5

Figure 3. Response surface experimental design using the central composite design (CCD) method. This design requires 11 trials composed of five levels of temperatures and humidity for Chinese cabbage storage, and is suitable for application to response surface methodology to comprehensively identify optimal storage temperature and humidity.



Pentagonal design				
experimental trial	Coded value		Uncoded value	
	x1	x2	Temperature(x1)	humidity(x2)
1	1.62	0.53	24.9	93.8
2	1	-1.38	20.0	89.1
3	0	0	12.0	92.5
4	0	1.70	12.0	96.8
5	-1	-1.38	4.0	89.1
6	-1.62	0.53	-0.9	93.8

Figure 4. Response surface experimental design using the equiradial (pentagonal) design method. This design focuses on rotatability, which is required for developing a 2^{nd} order response surface model with minimal trials.

Equiradial design

Equiradial design is a useful method for designing a two-variable experiment, which proposes a minimal number of experimental trials for RSM (Parker et al., 2006). This design is based on the principle that the second-order model requires a rotatable form; thus, the experimental points are determined from an inscribed polygon for the circle composed of the experimental points. This design should contain more than five points of contact and more than one center point for two variables, and is categorized as a hexagonal or pentagonal design depending on the number of points of contact. For example, we can set up a six-trial experiment composed of five and four levels of two variables. In the case of an experiment investigating changes in GSL levels in response to environmental conditions, we can design six measurement spots using the equiradial design with 4°C and 25°C as the low and high standard code temperatures. The range of humidity determined by temperature coding was from 89% to 97% (figure 4).

Conclusion

This study has reviewed notable literature on variations in the quality and functional components of Chinese cabbage under various storage conditions; additionally, the study proposed effective experimental designs based on response surface methodology. Overall quality measures such as color, firmness, and functional component levels (including GSLs) decreased during the storage of Chinese cabbage; however, the deterioration rates could be reduced by lowering storage temperatures and controlling atmospheric conditions. Three different proposed experimental designs introduce the number of experimental points, levels of storage conditions (temperature and humidity), and types of RSM models. Preserving the quality of Chinese cabbage has been highlighted as important because of the requirements for long-term storage of cabbage, and for maintaining the functional components of cabbage that are important for human health. For this reason, we expect that this study will provide some valuable information for designing storage conditions for Chinese cabbage that optimize the maintenance of functional components (including GSLs). For example, in future research, the comprehensive optimization of production and storage methods for Chinese cabbage containing maximized GSL is necessary to meet current demands for functional foods.

Conflict of Interest

The authors have no conflicting financial or other interests.

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