Mass Spectrometry-based Lipidomics for Freshness Markers Discovery in Cabbage

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（質量分析を用いたリピドミクスによるキャベツの鮮度マーカーの探索）

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I. INTRODUCTION

Quality is an important term in the postharvest chain and consumer. Notably, quality implies the character of the product, which is related to the degree of excellence of fresh product or absence of the defect started from harvest to consumer. Fresh produce such as fruits and vegetables have a high moisture content that occurs the increased metabolic activity. This activity continued after harvesting, and it affects short-shelf life due to degradation of quality.

Lately, the term freshness is most famous on the consumer, and it is a common word encountered in daily life. This term is quality criteria that have the optimal condition for acceptance by consumers. The freshness also can describe the condition quality of being natural and not preserved by a process such as freezing. On the chemist side, the freshness is related to oxidative changes, and on the physical side, it is related to changes in appearance or structure. The appearance and flavor are easy to perceive by human senses, but the nutritional component requires the equipment.

In daily life, the consumers judge the freshness from their senses such as sight, touch, smell. This fact, the consumer can estimate the freshness condition only from optical cues that tend subjectively (Péneau et al. 2007). Therefore, producers, distributors, and retailers should take into account the freshness of products. However, the assessment from appearance is entirely subjective, and the judgment result often varies depending on the evaluators. A freshness assessment providing quantitative results should be developed. To date, in the research field of post-harvest technology, the effectiveness of developed storage methods and distribution framework has been quantitatively evaluated by measuring multiple quality factors that decrease with time, such as ascorbic acid, sugar content, moisture content, and texture (Barth and Zhuang 1996; Medina et al. 2012; Hasperué et al. 2016). However, since these assessment methods are based on relative evaluation to the values at the time of harvest, it is difficult to

apply them to the freshness assessment in the actual distribution process, where the values at the time of harvest cannot be obtained retrospectively.

Several trials for developing the freshness assessment method based on physical and physiological changes during senescence have been done. For instance, chlorophyll fluorescence properties, which reflects the soundness of the photosynthesis system in the chloroplast, was related to storage duration and temperature and found to be one of the freshness indicators in spinach leaves and broccoli head (Toivonen and Deell 1998; Qiu et al. 2017). LED-induced fluorescence was also applied for measuring firmness and soluble solid content, which decline with aging in apple fruit, and these freshness parameters could be predicted by the partial least square regression model obtained from the fluorescence spectra excited by an LED light at 375 nm (Gao et al. 2016). Moreover, Raman spectroscopy employing a 532 nm laser for excitation was utilized for determining the carotenoids of citrus fruits as a freshness parameter (Nekvapil et al. 2018). The distribution characteristics of luminance in the image of cabbage and strawberry were used for freshness assessment since it is highly correlated with the perceived glossiness and lightness of the visual texture (Wada et al. 2010; Arce-Lopera et al. 2012, 2013).

However, these technologies are available for limited kinds of products, for example, the chlorophyll fluorescence method can be applied for fresh produce that contains chlorophyll. Also, they are based on the measurement of a specific quality parameter such as firmness, pigment, the nutritional value in spite that freshness must represent overall quality. Since freshness associates with the progress of senescence characterized by the loss of biomembrane integrity, focusing on the soundness of biomembrane would be better for assessing the freshness rather than measuring the quality parameters mentioned above.

Biomembrane is formed by a lipid bilayer that contains amphiphilic molecules. It plays essential roles, such as barrier function and membrane trafficking (Van Meer et al. 2008).

Furthermore, lipids act as an energy source for membrane biogenesis (Casares et al. 2019) and signaling molecules for transmitting information from outside (Xiong et al. 2002). Additionally, lipid has a broad diversity in species due to the variety of the number of carbons and double bonds in hydrophobic tail parts and structures in hydrophilic head parts (Nakamura 2017; Harayama and Riezman 2018). It has been reported that more than 10,000 kinds of lipid species exist in biomembrane (Sud et al. 2007), and living things maintain homeostasis by changing their composition in response to environmental stress (Barrero-Sicilia et al. 2017; Agmon and Stockwell 2017). Therefore, lipid profile involves useful information on biological status, including senescence.

Lipidomics is a subset of metabolomics focusing on lipids, and it is beneficial not only for understanding the biological mechanism but also for finding out the biomarkers based on the comprehensive analysis of lipids. Mass spectrometry-based lipidomics is a powerful technique that has a high sensitivity, resolution, and accuracy in measuring thousands of different lipid species. It has been utilized to elucidate physiological responses to various environmental stress such as chilling and drought in plants (Welti et al. 2002a; Mishra et al. 2006; Gasulla et al. 2013; Jia et al. 2013). Also, Lipidomics has been applied in food sciences, such as grading olives and authentication of almonds (Shen et al. 2013; Alves et al. 2019). Therefore, Lipidomics is expected to provide useful information on physiological reactions occurring in fresh produce during senescence. Furthermore, it would be able to elucidate the specific lipid species that indicate the degrees of freshness.

This study aimed to investigate the change of lipid profile in whole cabbage during storage and identify the freshness marker species utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based Lipidomics. Recently, Lipidomics using analytical instrumentation such as LC-MS/MS has introduced a powerful approach to identify biomarkers in postharvest sciences because this instrumentation has triple quadrupole and

MS/MS to classify specific and quantity of lipid. (Yunping and Reed 2014; Holčapek et al. 2018). Some studies have used LC-MS/MS for sphingolipids profiling such as sphingosine, sphingosylphosphorylcholine, ceramide-1-phosphate, Hexosylceramides, miscellaneous (Scherer et al. 2010) and have detected a class of fatty acids, sterols, glycerolipids, sterol in grape (Della Corte et al. 2015).

The whole cabbage was selected as a sample material because it is one of the common vegetables in the world, and the freshness is an important attribute due to its short shelf-life. Moreover, the quantitative freshness assessment method is strongly desired, especially in the fresh-cut fruits and vegetable industry. The raw material cabbage is often stored at low temperature for a long period, and its freshness is affected to the quality of the final fresh-cut product. For these reasons, the whole cabbage stored at different temperatures and durations was used for the widely targeted Lipidomics by LC-MS/MS to discuss the feasibility of the freshness assessment by lipid profiling in this study.

II. LITERATURE STUDY

2.1 Beneficial fruits and vegetables

Fruits and vegetables contain a rich source of vitamins, minerals, fibers, and phytochemicals that maintain human health. Phytochemical or plant chemical is the main actor to protect the cells from damage which strongly associated with chronic diseases such as diabetes, stroke, cardiovascular diseases, hypertension, and cancer. Many kinds of phytochemicals contain fruits and vegetables, i.e., flavonoids, antioxidants, carotenoids, anthocyanins, phenolics, alkaloids, terpenoids, glycosylates, saponins, and sulfides (Wargovich 2000; Zhang et al. 2015). Vitamin and minerals are important for the human diet, essential in biological activities for normal cellular functions. The classification of vitamins and minerals contained in fruit and vegetables show in Figure 1.

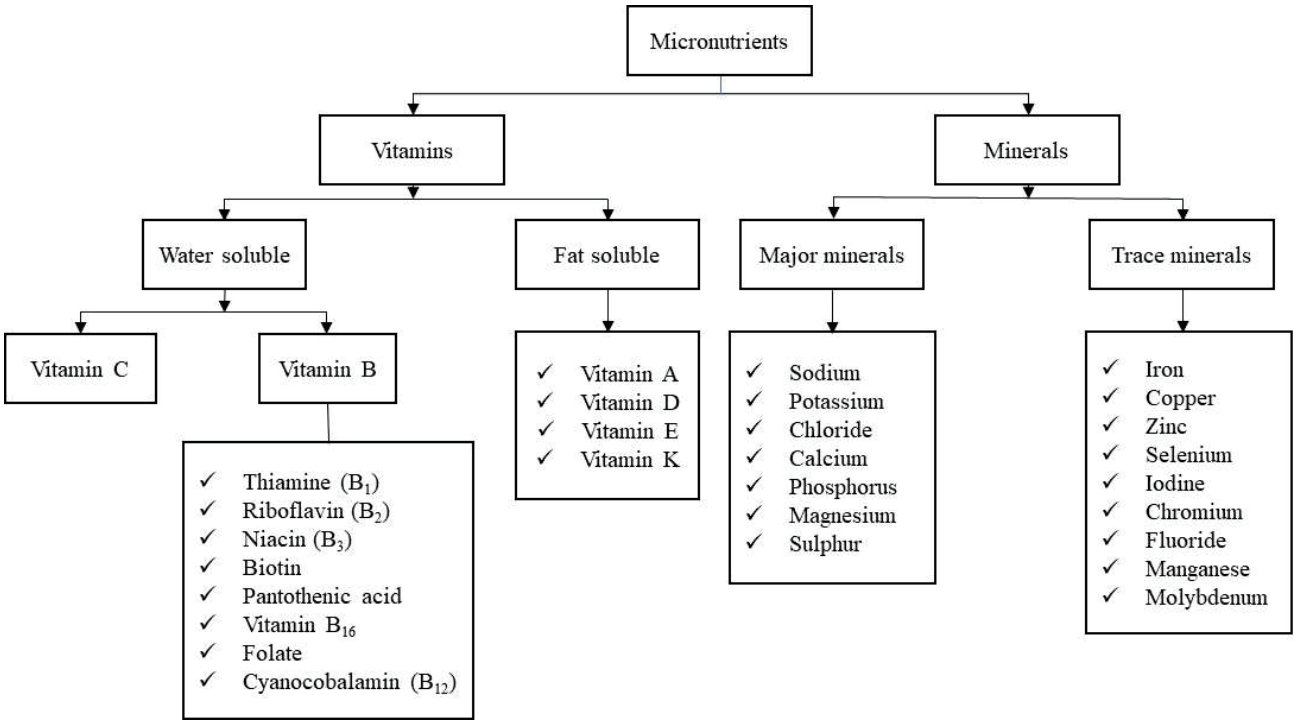


Fig. 1. Classification of micronutrients in fruit and vegetables.

Health authorities worldwide, such as the World Health Organization (WHO), promote to consume the high portion of fruits and vegetables. The overall daily consumption of fruit and

vegetables is 100 g underneath recommended intake (Amine et al. 2003). To fulfill the micronutrient and phytochemicals in the human body can be obtained from the high quality of fresh produce because their abundance differs in each product. Its abundance relates to the developmental stage of the product, which is influenced during pre-harvest and postharvest stages (Weston and Barth 1997; Parr and Bolwell 2000). The preharvest stage influences the quality changes in the postharvest stage because the quality of fresh produce during postharvest depends on the time of harvest. In addition, the external factor from preharvest, such as climate condition, cultivar variation, and cultural practices, can also affect the profile of produce. Therefore, an integrated approach is required to sustain the suitable level of micronutrient and phytochemicals in fresh produce from production, harvest, and postharvest until consumption.

2.2 Quality component to assess the freshness

Quality is the attribute that implies the degree of excellence of a product for a particular use. Generally, quality has some attributes such as sensory attributes (appearance, color, texture, flavor, and aroma), nutritive (primary metabolites, i.e., carbohydrates, amino acids, vitamin B complex, organic acids, fatty acids), and functional properties (secondary metabolites-phytochemical, i.e., flavonoids, phenolic acids, lignans, carotenoids, tocopherols, quinones, sterols, alkaloids, glucosinolates) (Hounsome, Hounsome, Tomos, & Edwards-Jones, 2008). Quality also interprets the human construct to comprise the properties or characteristics. Quality will interpret the product without defects that show a degree of excellence. The quality of fresh produce will change after harvesting because activities biology continues. The changes will affect the price and acceptance of consumers.

In judging the quality of attributes, Shewfelt (1999) divides into two perspectives, i.e., product-oriented quality and consumer-oriented quality. In the product-oriented quality views, quality is determined by the accuracy and precision of the measurement, and the alteration of quality also correlates with time and physiological changes. Researchers used this perspective

as a reference in evaluating postharvest technology, such as the effect of storage condition, handling system, quarantine treatment, and another postharvest variable. Consumer-oriented quality is needed to predict product performance in the marketplace. Understanding consumer behavior is utilized for assessing this performance. Consumers use all of their senses to evaluate quality, such as sight, smell, taste, touch, and even hearing. In a final judgment of fresh produce's acceptability, the consumer integrates all of those sensory in appearance, texture, aroma, and flavor of produce. From the perspective mentioned above, we can conclude that the combination of characteristics of the product itself be termed quality, and the consumer's perception and response to those characteristics be referred to as acceptability (Abbott 1999).

Freshness is an important term to describe the quality of fresh produce which no signs of withering or aging and have not deteriorated. Freshness becomes the main aesthetic factor that influences the consumer to decide on purchasing fresh produce. Freshness also reflects the nutritive value and phytochemical content in produce. Although, the degradation of freshness in fruits and vegetables, especially appearance attributes, gave the side effect to the appetite of consumers for purchasing. Péneau et al. (2007) suggest that appearance has a high impression at first sight of consumers and the most important component to acceptance and purchase decision. Péneau, Hoehn, Roth, Escher, & Nuessli (2006) described that the freshness assessment was determined from time after harvesting to the marketplace and finally consumed by the consumer. The freshness can represent all quality attributes and assesses the high-quality category. The common quality attribute to assess freshness is sensory attributes such as appearance, flavor, and taste. The easy observation is appearance because visual appearance can be observed by everyone in the distribution stage and marketplace (Figure 2).

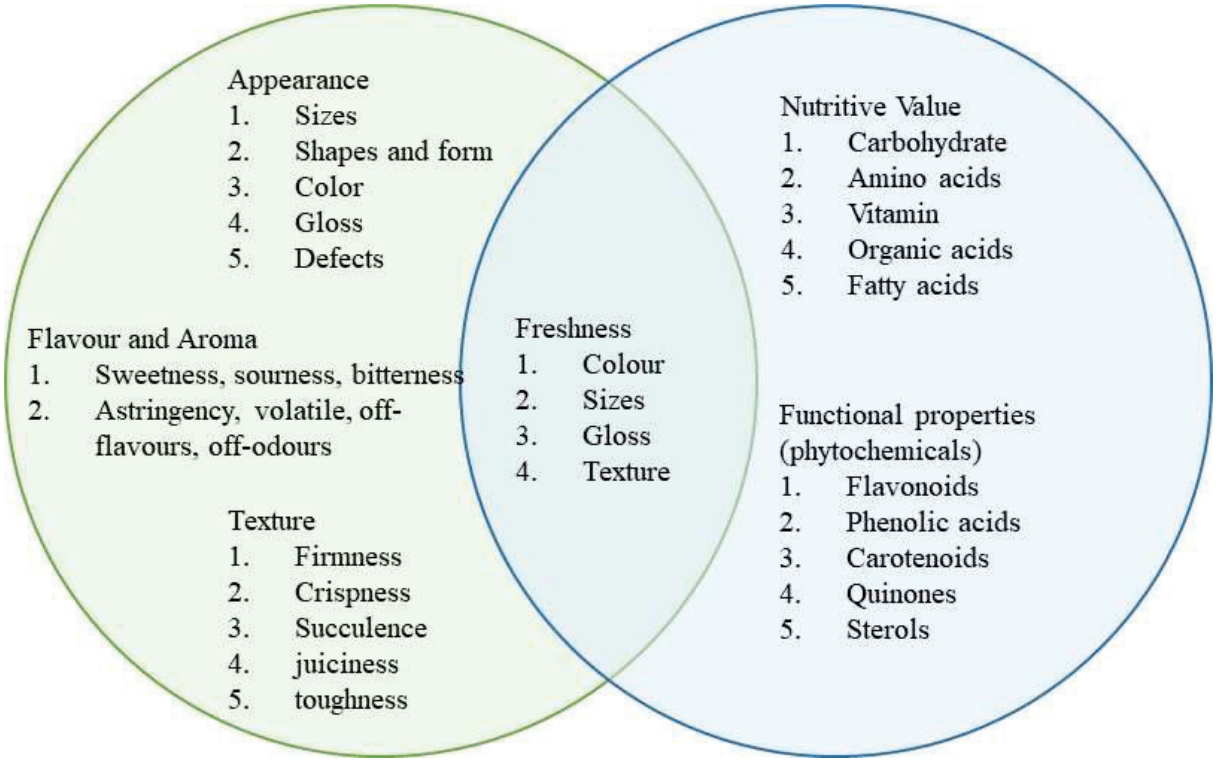


Fig. 2. The quality attribute of fruits and vegetables and consumer perception about freshness.

2.3 Application of mass spectrometry-based metabolomics for agricultural products

Metabolomics studies biological systems, focusing on metabolites, a broad range of small molecules (Johnson et al. 2016). This study can provide information about the cellular state of living things. Generally, metabolomics elucidates chemical processes in biological samples and discovers biomarkers through profiling metabolites. Recently, agricultural research has utilized metabolomics to understand the physiological and biochemical changes under stress conditions such as temperature (Kaplan et al. 2004; Johnson et al. 2016), drought (Gundaraniya et al. 2020), salinity (Shelden et al. 2016), etc. Food research also uses it to assure food quality (Guo et al. 2012), safety (Parlapani et al. 2015), and traceability (Garrett et al. 2013).

"Postharvest" is the handling of agricultural products such as fruits, vegetables, legumes, spices, and nuts after they are separated from the parent plant. During this stage, they continue metabolic activity associated with physiological and biochemical changes. To evaluate these

changes, respiration, ethylene production, transpiration, total soluble solids, soluble sugars, starch, and pigments have been measured. However, a conventional analysis provides little information on the physiological status of postharvest agricultural products because it covers only limited, specific parameters.

Alternatively, metabolomics produces multitudes of data regarding the physiological and biochemical changes during the lifespan of agricultural products. Understanding postharvest physiological changes leads to improved postharvest technology such as maintaining and assuring the quality of agricultural products (Malheiro et al. 2013; Corpas Iguarán et al. 2018). Additionally, discrimination of species, cultivar, and geographic origin verified via metabolic biomarkers will detect counterfeit products and certify quality (Lamanna et al. 2011; Kim et al. 2020). In recent years, several researchers have tried to apply the metabolomics approach to clarify the mechanism of ripening, senescence, and disorders (Aizat et al. 2014; Xu et al. 2018b). Moreover, it has been used to discern species or cultivars (Malheiro et al. 2013) and the geographic origin of the product (Oh et al. 2019; Khakimov et al. 2022).

2.3.1 Metabolomics for understanding on physiological alterations

After harvesting, fruits and vegetables continue a biological activity. These metabolic changes affect their quality. Especially in climacteric fruits, ripening is important to achieve optimum quality. Because fruits are not of optimum quality at harvesting time, ripening continues in postharvest until fruits are ready to eat. In ripening, drastic metabolic changes occur, leading to quality improvement. Senescence is also an important physiological phenomenon affecting quality. Complex metabolic shifting occurs in this stage. Understanding ripening and senescence mechanisms is needed for maintaining and assuring the quality of fresh produce. Metabolomics is one solution and has recently become popular for understanding physiological changes and disorders during ripening and senescence in fresh produce as shown in Table 1.

Uneven ripening at harvest causes delivery of inconsistent quality to consumers. Uniformity attracts consumers and guarantees high quality. To address this problem, researchers have applied metabolomics to understand the ripening mechanism in fruits. Parijadi, *et al*. (Parijadi et al. 2018) found that psicose, fructose, xylose, and other 13 compounds become metabolic markers enabling description of the degree of mangosteen ripening by using GC-MS metabolomics. Similarly, Pedreschi, *et al*. (Pedreschi et al. 2014) applied untargeted multiplatform metabolomics using both LC-MS and GC-MS to reveal the heterogeneity of ripening stages of "Hass" avocado. In their experiment, avocado samples were separated into five clusters based on time to ripeness (9 days, 13 days, 17 days, 20 days, and >22 days). Amino acids such as glutamic acids, aspartic acids, alanine, and galacturonic acid contributed to the ripening of "Hass" avocado. Particularly, glutamic acids, aspartic acids, and alanine were detected between fast and slow ripening clusters while galacturonic acid was only detected in the fastest ripening clusters. This observation indicates that metabolic profiling can elucidate the heterogeneity of "Hass" avocado ripening stages.

In kiwifruit, exogenous ethylene treatment is commonly used as artificial ripening to accelerate the process because these fruits need a longer ripening time. Qualitative metabolite analysis is needed to investigate the differences of quality between normal ripening (NR) and exogenous ripening (ER). This work noted that concentrations of sucrose, myo-inosistol, citric acid, and malic acid were higher in NR than ER fruits and fructose, glucose, and quinic acid were higher in ER fruits. This observation indicated that ethylene treatment in kiwifruit during storage can help the ripening and result in good quality (Lim et al. 2017). The potential of metabolomics has also been exhibited in non-climacteric products such as capsicum (Aizat et al. 2014), pineapple (Ikram et al. 2020), and cherry (Karagiannis et al. 2018). The common non-climacteric fruits are not ripe after harvesting, but several commodities such as capsicum have a unique ripening behavior. For instance, the field ripening stages of capsicum are

classified into seven groups, i.e., green, deep green, breaker, breaker red, bright red, deep red, and deep red + dried. Harvesting at the green and deep green stages results in failure of full ripening. Breaker red stages are the best time to harvest. Capsicum fruits harvested in this stage can develop to the fully red stages during storage (Krajayklang et al. 2000). In this regard, Aizat, *et al*., (Aizat et al. 2014) utilized GC-MS and LC-MS to elucidate the unique ripening mechanism in capsicum. GC-MS was used for screening potential markers among the ripening stages, and then, LC-MS was applied to enhance the characterization of selected metabolites. From the metabolic profiling in capsicum, the modification of sugar, amino acids, organic acids, and polyamines was found during ripening. These results highlighted the fundamental role of metabolites in renewing the grading method in non-climacteric products.

Flavor is an essential attribute to evaluate senescence and is a combination of aroma and taste. The shifting of flavor depends on fruit type. Sugars, acids, and volatiles produce and alter fruit flavor (Gonçalves et al. 2018; Pott et al. 2020). Iguaran, *et al*. (Corpas Iguarán et al. 2018) conducted a comprehensive volatile analysis to clarify the causes of off flavor during senescence in lulo fruits. In this study, headspace-solid phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS) was applied, and 15 volatile compounds were found to be potential markers of off flavor. Particularly, methyl ester was dominant in lulo fruit senescence. In citrus fruits, flavor is also used as a reference to assess senescence. Sun, *et al*. (Sun et al. 2013) applied metabolomic profiling using GC-MS to understand the metabolic role in organic acid changes. Profiling highlighted that succinic acids, γ-aminobutyric acid (GABA), and glutamine increased, whereas 2-oxoglutaric acid decreased during orange senescence. The postharvest phenomenon from ripening to senescence in pear was captured via metabolic profiling. The degradation of 18 species of sugars such as D-fructose-6-phosphate, arabinose, and trehalose 6-phosphate did not affect pear softening during senescence, whereas glycerophospholipids did (Xu et al. 2018a).

Storage temperature affects enzyme activity, which induces metabolite changes in fresh produce. Inadequate postharvest storage conditions not only influence ripening and senescence but also result in physiological disorders. Chilling injury (CI) occurs in chilling sensitive products under low temperatures. CI is caused by membrane damage due to lipid peroxidation. The imbalance of respiratory and other metabolic processes accelerates the occurrence of lipid peroxidation (Repetto et al. 2012; Saltveit 2019). The common symptoms of CI are pitting, failure of ripening, internal discoloration, water soaking, and browning (Wang 2010). Metabolomics gave a satisfying result for investigating the metabolic alteration caused by CI. Cozzolino, *et al*. (Cozzolino et al. 2016) used GC-MS with HS-SPME to investigate the profile of volatile compounds in basil leaves and found 1,8-cineole was a volatile marker to diagnose the early symptoms of CI. Identification of metabolomic changes also provided an understanding of the CI tolerances in mangoes. This understanding provided knowledge about the function of antioxidant and phenolic levels in suppressing cell damage by attacking reactive oxygen species (Vega-Alvarez et al. 2020). Apple (var. *domestica* Borkh. Mansf.) is susceptible to necrosis characterized by superficial scald. This physiological disorder is induced by cold storage. A metabolomics approach identified phytosterol metabolism as responsible when superficial scald occurred (Rudell et al. 2011).

2.3.2 Metabolomics for agricultural products authentication

Food adulteration is illegal worldwide but continues even now. Adulteration occurs when the authentic substance is replaced with a cheaper one to increase the volume and weight of the product for financial gain (Danezis et al. 2016). Because it threatens food reliability, researchers have worked to develop methods to detect food adulteration, especially for expensive products like honey and meat. Honey is popular among consumers because of its health benefits but is expensive, making it a prime target for adulteration. Adding a cheaper sweetener like corn syrup or inverted sugar syrup is a common trick to increase profits (Xue et

al. 2013). Similarly, minced beef adulterated with other meat, like pork, is often found in the market because of high beef demand (Trivedi et al. 2016). In this case, metabolomics has been used to identify food adulteration (Xue et al. 2013; Trivedi et al. 2016).

Additionally, the metabolomics method efficiently verifies the true composition from different species, varieties, and origins. It is essential to certify authenticity to protect economic value (Lamanna et al. 2011; Ikram et al. 2021). Nowadays, metabolomics authentication has been utilized in agricultural products to ensure quality, establish the brand, and avoid false claims of origin, species, and variety. Tables 2 and 3 show representative examples of metabolomics used to discriminate or distinguish the agricultural product of different species, varieties, and origins.

2.3.2.1 Discriminant of species, varieties and cultivar

Discrimination by appearance among different species of agricultural products like tea leaves, coffee, and some fruits and vegetables is often challenging. This difficulty leads to adulteration and threatens the validity of a quality guarantee. The quality of several products is characterized by their unique aroma and taste. Commonly, these aromas and tastes are assessed by sensory evaluation (Abbott 1999). However, this evaluation is inefficient because evaluator training is costly and an untrained organoleptic evaluator produces subjective results. Metabolomics can overcome this problem by detection and quantification of specific biomarker metabolites that accurately discriminate between species and varieties. Moreover, the metabolic profile aids understanding of product characteristics that are useful to enhance the product brand.

For instance, mushroom is a multipurpose fresh product used for food and pharmaceuticals. Aroma is an essential attribute in mushrooms as each cultivar has a unique aroma correlated with quality. Malheiro, *et al*. (Malheiro et al. 2013) used HS-SPME-GC-MS metabolomics to define six species of wild mushrooms on the basis of their volatile compounds. Untargeted

analysis was followed by targeting 46 volatiles to discover the discriminant markers. From this report, the volatile profile accurately described each species on the basis of their unique aroma. Eleven volatile compounds were fundamental to distinguish mushroom species. For example, 3-octanol and 1-octanol were important for *L.nuda* species, linalool for *T.fracticum*, and 3-octanone for *H.crustuliniforme*.

In tea, taste is an essential attribute for consumers. Taste differences are characterized not only by varieties but also by subtypes. Metabolite diversity affects the specific taste of tea. Yang, *et al*. (Yang et al. 2018) utilized ultrahigh performance liquid chromatography–time of flight/mass spectrometry (UHPLCQ-TOF/MS) to characterize the taste of different Chinese white tea subtypes, namely, Silver Needle, White Peony, and Shou Mei. Metabolomics identified 99 non-volatiles, and clarified that theanine, aspartic acid, asparagin, and AMP were responsible for the umami taste. Additionally, they found that flavan-3-ols, theasinensins, procyanidin B3, and theobromine were responsible for bitterness and astringency. Monti, *et al*. (Monti et al. 2016) conducted metabolic profiling to elucidate the chemical biodiversity of 15 peach varieties at different ripening stages. This study demonstrated that specific metabolites involving the organoleptic and nutritional properties depended on peach variety. Six varieties were characterized by fructose and glucose, and two varieties were characterized by malic and citric acid.

To several consumers, mango has an exotic taste. Worldwide, there are approximately 350 commercial cultivars, each with a unique taste. Sato, *et al*. (Sato et al. 2021) investigated metabolites in mango characterizing the taste of five cultivars from Indonesia. In this report, GC-MS identified 95 metabolites of interest. Orthogonal projection to latent structure-discriminant analysis (OPLS-DA) separated these metabolites into three groups in the five cultivars. Nicotinic acid, glutamic acid, aspartic acid, glycine, and ribose contributed to the identification. These results highlighted the potential role of biomarker metabolites that could

be commercially useful for sorting mango varieties. Similarly, taste is crucial in pineapple, and metabolic profiling by GC-MS revealed that GABA, valine, and alanine defined the 'Red Spanish' cultivar, sucrose, threonic acid, and 5-hydroxytryptamine (serotonine) defined 'Smooth Cayenne', and threonine, serine, and methionine defined 'Queen' (Ikram et al. 2021).

Honey is produced by honeybees from flower nectar. In New Zealand, Manuka honey from the species *Leptospermum scoparium* J.R is popular among consumers because of its health benefits but is costly. The cost makes Manuka honey a target for adulteration. Mixing Manuka honey and Kanuka honey is a common way to increase profits. Metabolic profiling of honey using UHPLC-PDA-MS/MS for non-volatile and HS-SPME-GC/MS for volatiles discriminated clearly among Manuka, Kanuka, and Jelly Bush honey. Leptosin, acetyl-2-hydroxy-4-2-(2-methoxyphenyl)-4-oxobutanate, 3-hydroxy-1-(2-methoxyphenyl)-penta-1,4,-dione, kojic acid, and 5-methyl-3furancaerboxylic acid were found to be biomarkers in Manuka honey (Beitlich et al. 2014). Additionally, the utilization of biomarkers elucidated by metabolomics using GC-MS distinguished wine produced from four grape varieties. In this study, 120 commercial brands representing four varieties from six different countries were used for analysis. Organic acids and sugar are dominant metabolites in all wine; however, serine, phenylalanine, L-homoserine, and glutamic acid were the best biomarkers in discriminating between the four varieties (Khakimov et al. 2022).

2.3.2.2 Discrimination of geographical origin

Consumers' concern over quality certification of agricultural products regarding the geographical origin is increasingly common for coffee and tea (Diboun et al. 2015; Kim et al. 2020; Rivera-Pérez et al. 2021). Traceability of geographical origin is usually used to protect the product from fraud. Recently, the European Union and the United Kingdom introduced protected designation of origin (PDO) and protected geographical indication (PGI) classifications. PDO or PGI aims to protect the original product from adulteration. PDO and

PGI products are registered in a list of quality products to trace their specifications (Florkowski, Wojciech et al. 2014; Mahajan et al. 2017). Because of this trend, researchers are motivated to develop advanced technology for discerning geographical origins. The metabolomics approach using MS and NMR is a powerful tool for authenticating several agricultural commodities.

Coffee is a popular beverage brewed from roasted coffee beans. The most common coffee beans used all over the world are *C. arabica* and *C. robusta*. Recently, the global coffee market is more concerned with aroma and taste. Different coffee origins produce specific aromas and tastes. In this context, HS-SPME-GC-MS comprehensively detected metabolites from samples of different origins, namely, Ethiopia, Tanzania, and Guatemala. The metabolic profiles from different origins were successfully separated using principal component analysis (PCA). Ethiopian coffee aroma was characterized mainly by 4-(4′-hydroxyphenyl)-2-butanone (Akiyama et al. 2008). In Indonesia, coffee production is spread over islands such as Java, Sumatra, Sulawesi, Bali, and Papua. Every island has several coffee production areas. Particularly, Mandheling is famous in Sumatera, Toraja in Sulawesi Island, and Kintamani in Bali. They are well known in the global market. Different coffee bean origins result in unique metabolites. The metabolomics approach is a valuable method to distinguish the origin of coffee beans. For instance, a comprehensive analysis for metabolic profiling of primary metabolites using non-targeted GC/MS successfully identified 64 compounds in coffee beans from nine areas in Indonesia. PCA separated the metabolic profiles of each coffee bean into three groups, i.e., western, central, and eastern Indonesia. Glycerol, glucan-1,5-lactone, gluconic acid, sorbitol, galactitol, and galactinol were potential markers distinguishing different regions. As mentioned above, exhaustive profiling and specific biomarkers are more valuable in authenticating coffee bean origin than utilization of conventional analyses like the cupping test (Putri et al. 2019).

Similarly, Mi, *et al*. (Mi et al. 2021) demonstrated the ability of metabolomics to distinguish the origin of garlic. They applied HS-SPME-GC-MS and UHPLCQ-TOF/MS to identify both volatile and non-volatile compounds. GC-MS identified 68 volatile compounds and LC/MS detected 854 non-volatile compounds. Two ketones, one alkane, mequinol, and 2-Methoxyphenol contributed to the garlic origin discrimination.

Adzuki bean is usually used as an ingredient for traditional desserts in East Asia, such as Japan, Korea, and China. This bean is also famous in the global market and has become an export product. Metabolomics analysis is performed to discern the geographical origin of the adzuki bean to certify authenticity. GC-TOF/MS and OPLS-DA was utilized to investigate metabolite profile in adzuki beans from Korea and China. Malic acid and citric acid were important markers to distinguish adzuki bean production areas (Kim et al. 2020). In black pepper, using high-resolution mass spectrometry (HRMS) using orbitrap mass analyzer, fatty acid derivatives like 10,16-dihydroxyhexadecanoic acid were found to be a strong marker to distinguish black pepper from Brazil, Vietnam, and Sri Lanka (Rivera-Pérez et al. 2021).

2.4 Application of mass spectrometry-based lipidomics for agricultural products

The common quality attribute to assess freshness is sensory attributes such as appearance, flavor, and taste. The easy observation is appearance because everyone in distribution stage and marketplace can observe visual appearance. However, the comprehensive evaluation from sensory attributes, especially appearance, is challenging to get the precision result because the interpretation of freshness from this side is dependent on the evaluator. Since freshness associates with the progress of senescence characterized by the loss of biomembrane integrity, focusing on the soundness of biomembrane would be better for assessing the freshness rather than measuring the quality parameters. Biomembrane is formed by a lipid bilayer that contains amphiphilic molecules. It plays essential roles, such as barrier function, membrane trafficking, signalling, and energy source for membrane biogenesis. So that focusing on membrane lipid

serve the valuable information not only for understanding the biological mechanism but also for finding out the biomarkers based on the comprehensive analysis of lipids.

To date, the rapid development of different analytical techniques has made it possible to obtain a proper consideration of these lipids, and MS has been by far the dominating instrument for lipid analysis. The MS-based analysis is cardinal from all these procedures as datasets for lipid qualification and quantitation are generated in this segment. However, it cannot be neglected that suitable sample preparation and optimal parameter settings for data analysis are also prerequisites to ensure the quality of acquired data and final results. Due to the chemical complexity and wide concentration range of lipids found in biological samples, it is daunting to identify and quantify all lipids simultaneously with a single analytical strategy. Therefore, depending on the purpose of analysis, MS-based Lipidomics can be performed using target or untargeted approaches, each with its distinctive features, inherent advantages, and limitations. Typically, targeted MS-based lipidomics approaches are employed when defined lipids or lipid classes of interest needed to be characterized. In this regard, triple quadrupole (QqQ) MS with selective MS scanning modes such as neutral loss scanning (NLS), product ion scanning (PIS), and multiple reaction monitoring (MRM) is the most often used platform. It is characterized by high detection sensitivity, selectivity, and quantitative accuracy with good linearity and reproducibility. Under these scanning modes, specific lipid fragmentation ions can be screened to identify and quantify defined lipid species. For example, product ion at m/z 184 and NLS of m/z 141 in the positive-ion mode are often used to assign phosphatidylcholine (PC) and phosphatidylethanolamine (PE), respectively (Shen et al. 2012). As a result, targeted MS-based Lipidomics has been considered as the gold standard for lipid quantitation and is tailored for analyses of lipids in low concentrations or with specific structures, such as lipid oxidation products generated in Arabidopsis (Ito et al., 2017; Leung, Galano, Durand, & Lee, 2018). The

main drawback of this strategy is the limited lipid coverage, and targeted lipidomics approaches cannot be applied to pin down unknown lipids.

By contrast, untargeted MS-based Lipidomics is a newly emerged strategy for lipid analysis and enables coverage of most lipid classes in an unbiased way, making it an ideal platform to screen lipid markers for food authentication at a global level. For this purpose, high-resolution MS (HRMS) such as time-of-flight (TOF), orbitrap, or Fourier transform ion cyclotron resonance (FTICR) is often employed due to its high mass resolution (>10, 000) and mass accuracy (<2–5 ppm). In recent years, quite a lot of untargeted lipidomics approaches based on HRMS, especially the TOF-MS and orbitrap, have been developed and applied for untargeted lipid analysis in different food matrices, including fish (Zhang et al., 2018), meat (Mi et al., 2019) and nuts (Song et al., 2018). In the untargeted MS-based lipidomics analyses, two main MS/MS data acquisition modes are data-dependent acquisition (DDA) and data-independent acquisition (DIA). In DDA, only precursor ions from a full MS scan that meets specific requirements, usually exceeding specified intensity thresholds, are selected for fragmentation. An obvious limitation of this mode is the incomplete lipid coverage. As an alternative to DDA, DIA has been recently developed for untargeted Lipidomics and uses defined windows to acquire MS/MS spectra of all precursor ions simultaneously. One of the most widely applied techniques for DIA is the sequential window acquisition of all theoretical fragment ion spectra (SWATH) introduced by Gillet et al. in 2012.

Table 1 Applications of metabolomics approaches for understanding the physiological changes and disorders

Purpose	Fresh produce	Analytical platform	Multivariate Analysis	Biomarkers	References
Ripening stages	Mangosteen	GC-Q	PCA, PLS, HCA	Psicose, fructose, xylose, galacturonic acid, and glucose are markers from the pericarp part. Xylose, xylulose, ribulose, glucuronate, 2-aminoisobutyric, and tryptophan are markers from the flesh part. Phenylalanine, valine, isoleucine, serine, tyrosine are markers from seeds part	(Parijadi et al. 2018)
	Avocado	LC-QTOF GC-TOF	PCA	Glutamic acids, aspartic acids, alanine, and galacturonic acid	(Pedreschi et al. 2014)
	Kiwifruits	GC-Q	PCA, PLS-DA	Sucrose, myo-inositol, citric acid, malic acid, fructose, glucose, and quinic acid	(Lim et al. 2017)
	Pineapple	GC-Q	PCA,OPLS-DA, PLSR	Melezitose, inositol, xylonic acid, gluconic acid, raffinose are markers from flesh part. Inositol, mannose, galactose, sucrose, aspartic acid are markers from the peel part.	(Ikram et al. 2020)
Senescence stages	Pear	LC-QqQ	PLS-DA	LysoPC, 16:0, 18:1, 18:2, 18:3, lysoPE 16:0, 18:2, MAG 18:2, 18:3, 18:4, punicic acid, 9-hydroxy-(10E, 12Z, 15Z)-octadecatrienoic acid, and 4-hydroxysphinganine	(Xu et al. 2018a)
	Lulo	GC-Q	PLS-DA	Methyl (E)-2-butenoate, 4-heptanone, o-xylene, (Z)-3-hexenyl acetate, hexyl acetate, 3,7-dimethyl-1,6-octadien-3-ol, the alcohols 1-penten-3-ol, (E)-3-hexen-1-ol, pentanal aldehyde, and 1,7,7-trumethyl-bicyclo[2.1.1]-heptan-2-one ketone	(Corpas Iguarán et al. 2018)

Table 1 (continued)

Purpose	Fresh produce	Analytical platform	Statistical Analysis	Biomarkers	References
Disorders during storage	Mango	LC-Q GC-QqQ	PCA	Galloylquinic acid, gallic acid esters, gallotannins, malic acid, citric acid, glucose, myo-inositol, and linoleic acid	(Vega-Alvarez et al. 2020)
	Basil	GC-Q	PCA, PLS	1,8-cineole, (Z)-β-ocimene, 1-hexanol, (E)-3-hexen-1-ol, 1-octanol, α-guaiene, α-Terpineol, bicyclogermacrene, hexanal, and (E)-2-hexanal	(Cozzolino et al. 2016)

GC-Q: gas chromatography-quadrupole; LC-Q: liquid chromatography-quadrupole; LC-QTOF: liquid chromatography-quadrupole time-of-flight; GC-TOF: gas chromatography time-of-flight; LC-QqQ: liquid chromatography-triple quadrupole; PCA: principal component analysis; PLS: partial least square; PLS-DA: partial least squares-discriminant analysis; OPLS-DA: orthogonal projections to latent structures modelling discriminant analysis; PLSR: partial least-square regression.

Table 2 Representative applications of metabolomics approaches for differentiation of species, varieties, and cultivars

Fresh produce	Analytical platform	Multivariate Analysis	Biomarkers	References
Wild mushrooms	GC-IT	PCA	3-octanol, 3-octanone, linalool, 1-octanol, 1-pentanol, (E)-2-octenal, and ρ-anisaldehyde.	(Malheiro et al. 2013)
Grape	GC-TOF	PCA, PLS-DA,	Serine, phenylalanine, L-homoserine, and glutamic acid	(Khakimov et al. 2022)
White tea	LC-Q-TOF	PCA, PLS-DA, HCA	Theanine, aspartic acid, asparagine, AMP, flavan-3-ols, theasinensisns, procyanidin B3, theobromine, epigallocatechin gallate, epicatechin, gallate, theogallin, and GABA	(Yang et al. 2018)
Peach	GC-Q	PCA	Fructose, glucose, malic acid, and citric acid	(Monti et al. 2016)
Mango	GC-Q	PCA, OPLS-DA	Nicotinic acid, glutamic acid, aspartic acid, glycine, ribose	(Sato et al. 2021)
Honey	GC-Q LC-QqQ	PCA, HCA	Leptosin, acetyl-2-hydroxy-4-2-(2-methoxyphenyl)-4-oxobutanate, 3-hydroxy-1-(2-methoxyphenyl)-penta-1,4,-dione, kojic acid, and 5-methyl-3furancaerboxylic acid	(Beitlich et al. 2014)

GC-IT: gas chromatography-ion-trap; GC-TOF: gas chromatography time-of-flight; LC-Q-TOF: liquid chromatography-quadrupole time-of-flight; GC-Q: gas chromatography-quadrupole; LC-QqQ: liquid chromatography-triple quadrupole; PCA: principal component analysis; PLS: partial least square; PLS-DA: partial least squares-discriminant analysis; OPLS-DA: orthogonal projections to latent structures modelling discriminant analysis; HCA: hierarchical cluster analysis.

Table 3 Representative applications of metabolomics approaches for distinguishing the geographical origin

Fresh produce	Analytical platform	Multivariate Analysis	Biomarkers	References
Adzuki beans	GC-TOF	OPLS-DA	citric acid and malic acid	(Kim et al. 2020)
Black pepper	LC-Q-Orbitrap	PCA, OPLS-DA	Reynosin, artabsinolide D, tatridin B, (12E)-9,10-Dihydroxy-12-octadecenoic, sitostenone, 9-hydroperoxy-10E-octadecenoic acid, 10,16-dihydroxyhexadecanoic acid, and 9,10-dihydroxystearic acid	(Rivera-Pérez et al. 2021)
Dates fruit	LC-FT GC-TOF	PCA, OPLS-DA	Ethanolamine, GABA, serotonin, tyramine, tryptamine, phenethylamine, serine, glutamate, tyrosine, tryptophan, phenylalanine, riboflavin, niacin, pyridoxine, and nicotinate	(Diboun et al. 2015)
Brewed Arabica coffee	GC-Q	PCA	(4′-hydroxyphenyl)-2-butanone	(Akiyama et al. 2008)
Coffee beans	GC-Q	PCA	Alanine, 4-aminobutyric acid, glycolic acid, quinic acid, lactic acid, pyroglutamic acid, malic acid, and caffein are Arabica coffee bean markers. Glycerol, glucono-1,5-lactone, gluconic acid, sorbitol, galactitol, and galactinol are markers from Robusta coffee beans.	(Putri et al. 2019)
Garlic	GC-Q LC-Q-TOF	PCA, PLS-DA	Benzene, 1,2-dimethoxy, ethenone, 1-(2-methyl-1-cyclopenten-1yl), mequinol, phenol, 2-methoxy, heptafluorobutyric acid, and n-tetradecyl ester	(Mi et al. 2021)
Asian palm civet coffee	GC-Q	PCA, OPLS-DA	Citric acid, malic acid, and inositol	(Jumhawan et al. 2013)
Hazelnut	LC-QqQ	PCA-LDA	PC 18:2/18:2, 16:0/18:2, 18:1/18:2, 16:0/18:3, 18:2/18:2, PE 18:2/18:2, 16:0/18:2, 18:1/18:1, DG 18:1/18:1, 18:2/18:2, 16:0/18:1, 16:0/16:1, TG 14:0/16:0/18:1, 15:0/16:0/18:1, 16:0/16:1/18:1, 17:1/18:1/18:2, and 18:2/18:2/18:3	(Klockmann et al. 2017)

GC-TOF: gas chromatography time-of-flight; LC-Q-Orbitrap: liquid chromatography couple-quadrupole-Orbitrap; LC-FT: liquid chromatography-Fourier transform; GC-TOF: gas chromatography-time-of-flight; GC-Q: gas chromatography-quadrupole; LC-Q-TOF: liquid

chromatography-quadrupole time-of-flight; LC-QqQ: liquid chromatography-triple quadrupole; PCA: principal component analysis; PCA-LDA: linear discriminant analysis based on PCA scores; PLS-DA: partial least squares-discriminant analysis; OPLS-DA: orthogonal projections to latent structures modelling discriminant analysis.

Table 4 Application Of MS-based Lipidomics in agriculture

Application	Lipid Source	Result	**Literature**
Geographical origin	Almonds	The method serves excellent promise for the selective separation of phospholipids from non-phospholipids, especially glycerides, and excels in rapid screening and characterization of phospholipids in almond samples.	Shen et al. (2013)
	Arabica Coffee	Success characterizes pure roasted coffee samples and coffee blends with 10% Robusta coffee added to Arabica coffee.	Cossignani, Montesano, Simonetti, & Blasi (2016)
	Tobacco leaves	Distinguishing fresh tobacco leaves based on geographical origin	L. Li et al. (2015)
	Banana	Comparing banana lipidomes by origin	Sun et al. (2020)
Adulteration	Goat milk, soymilk, bovine milk	A total of 14 lipids were identified as biological markers for milk type differentiation, thus providing the basis for milk authentication and adulteration detection.	Q. Li et al. (2017)
	Extract virgin olive oil, hazelnut oil	The method could separate adulterated extra virgin olive oil with hazelnut oil to the level of 1%.	Calvano, De Ceglie, D'Accolti, & Zambonin (2012)
	Wheat	Distinguishing between pure and blended durum wheat	Righetti et al. (2018)
	Goat, Sheep, Cow, Camel milk	Milk adulteration detection	Piras et al. (2021)

Table 4 Continue

Application	Lipid Source	Result	**Literature**
Processing and storage	African walnut	Impact of postharvest process on the fatty acid profile of African walnut	Nkwonta, Alamar, Landahl, & Terry (2016)
	Peanut oil	Differentiating the nutrition of 10 types of vegetable oil after optimal cooking optimal	Cui, Hao, Liu, & Meng (2017)
Varieties dan cultivar	Coconut oils	differentiating varieties of coconut oil	Ferreira et al. (2019)
	Cereal	Distinguishing seven types of cereals	Hammann et al. (2019)
Chilling stress	Paprika	Understanding the mechanism of cell damage during chilling injury	Kong et al. (2018)
	Banana	Understanding the mechanism of deterioration of membrane lipid during chilling injury	Liu, Li, Chen, & Jiang (2020)
Freezing stress	Arabidopsis	Understanding the response of lipid membranes under freezing stress	Welti et al. (2002)
Heat stress	Arabidopsis	Understanding the process of membrane damage under heat stress	Higashi et al. (2015), Shiva et al. (2020)

III. MATERIAL AND METHOD

3.1 *Plant material and storage condition*

The cabbage (cv. 'Asashiho') was harvested at the farmer's orchard in Ibaraki, Japan, in July 2020 and transported to the laboratory by the refrigerated delivery service in a day. Immediately after delivery, the cabbages were selected based on size uniformity and the lack of wound symptoms. The selected cabbages were put in a foamed polystyrene box and stored at 5 °C, 10 °C, and 20 °C in an incubator for 8, 4, and 3 weeks, respectively. Samples for the lipid analysis were collected individually from 5 different cabbage heads every one week. In sampling, the first and second outer leaf was removed, and the sample leaf disk was excised from the third outer leaf using a cork-borer with 3 mm in diameter. Subsequently, approximately 100 mg of disks were put into a 2 ml cryotube with two pieces of zirconia ball with 5 mm in diameter. Then, it was rapidly frozen by liquid nitrogen and stored at -80 °C until lipid analysis.

3.2 Measurement of respiration rate

The respiration rate of cabbage was measured by a flow-through method using gas chromatography (GC) as described in Fahmy and Nakano (2014) with some modifications. First, approximately 500 grams of cabbage were placed into an acrylic chamber (4.8L) with a gas inlet and outlets tube. The chamber was placed in an incubator which set at 5 °C, 10 °C, and 20°C, and the fresh air was flowed into the chamber using an air compressor through the inlet tube at a flow rate of 100 ml min^{-1}. The gas flow rate was controlled using a mass flow controller (SEF-E40, Horiba, Japan). Then, the inlet and outlet gas sample were injected automatically into GC (GC-14A Shimadzu, Kyoto, Japan) via 0.5 ml sampling loop attached to a rotating valve. CO_2 in the sample gas was separated using a Porapak Q column, and O_2 and N_2 were separated using the Molecular Sieve-5A column. Subsequently, these gaseous were detected by a thermal conductivity detector and analyzed using GC-Solution software

(Shimadzu, Kyoto, Japan) based on a calibration curve. The rate of CO_2 production was calculated based on differences between the outlet and inlet using equation (1) as described by (Fonseca et al. 2002).

$$R_{CO_2} = \frac{y_{CO_2}^{out} - y_{CO_2}^{in}}{100} \; x \; \frac{F}{W} \; x \; \frac{P}{RT} \; x \; 10^3$$

Where R_{CO_2}, is the respiration from CO_2 production of the sample (mmol kg^{-1} h^{-1}), y_{CO_2} is volumetric concentration in inlet and outlet (%), *W* is the weight of the sample (kg), *F* is the flow rate (ml h^{-1}), *P* is the atmospheric pressure (= 101.3 kPa), *R* is the universal gas constant (= 8.3141 J K^{-1} mol^{-1}) and *T* is the absolute temperature (°K).

3.3 Ascorbic Acid Analysis

Ascorbic acid (AsA) was measured using liquid chromatography method as described by Thammawong et al. (2019) with some modifications. Firstly, the frozen sample leaves were cryogenically ground in a bead crusher (ShakeMaster® NEO, Biomedical Science, Tokyo, Japan) using three of 5 mm diameter zirconia balls for 3 min at 1,500 rpm. Then, 100 mg of sample powder was put into 2 ml Eppendorf tube with 1.5 ml of 5% metaphosphoric acids. The mixture was vortexed for 1 minute and subsequently centrifuged at 20,000 × g and 4 °C for 15 minutes (Model 1720, Kubota Corp., Tokyo, Japan). Afterward, 500 µl of supernatant was collected and 50 µl of Tris (2-carboxyethyl) phosphine hydrochloride was added to reduce dehydroascorbate to L-AsA. The mixture was continuously shaken using a block bath shaker (MyBL-100SC, As One, Osaka, Japan) set at 2500 rpm and 25 °C for 25 minutes in a dark place.

A HPLC system (UltiMate 3000, Thermo Fisher Scientific, Massachusetts, USA) equipped with a polymer amino column (Asahipak NH2P-50 4E, 250 × 4.6 mm i.d., 5 µm in particle size, Shodex, Tokyo, Japan) was utilized to determine AsA concentration. Before measurement, the column was equilibrated by flowing 60 mM phosphoric acid at 0.5 ml min^{-1} for 2 hours. A mixture of acetonitrile / 20 mM $NaH_2PO_4O_6$ + 30 mM H_3PO_4 (80/20, v/v) was

used as a mobile phase and flowed at 1.0 ml min^{-1}. Just prior to injection, AsA extraction was diluted 10 times by Milli-Q water automatically using an in-needle mixing function of the autosampler, and then 10 µl of diluted sample was injected into the system. The column temperature was kept constant at 35 °C. The chromatograms were recorded at 244 nm using the UV detector. From the equation of the calibration curve plotted for the standard solutions, the concentration of AsA was estimated.

3.4 Lipid analysis by LC-MS/MS

3.3.1 Reagents

LC-MS/MS grade methyl-tert-butyl ether (MTBE), isopropanol, acetonitrile, chloroform, methanol and acetic acid were obtained from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). LC-MS/MS grade ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphatidylcholine (PC) 17:0/17:0 and phosphatidylethanolamine (PE) 17:0/17:0 were purchased from Avanti Polar Lipid, INC (USA) and used as internal standards.

3.3.2 Lipid extraction

Lipid was extracted using an MTBE method described by Matyash et al. (2008) with some modifications. Frozen sample leaf disks were cryogenically ground in a bead crusher (ShakeMaster® NEO, Biomedical Science, Tokyo, Japan) for 1 min at 1,500 rpm. Approximately Fifty milligrams weighed sample powder was put into 2 ml Eppendorf tube with 1000 µl of MTBE, 300 µl of methanol, and 50 µl of internal standard (20 µg ml^{-1} of each of PC 17:0/17:0 and PE 17:0/17:0 dissolved in a mixture of chloroform, methanol, and H_2O (6/4/0.5, v/v/v) and mixed thoroughly by a vortex mixer for 1 min). In dark conditions, the homogenates were shaken in a water bath set at 25 °C for 1 h at 100 rpm. Afterward, 250 µl of H_2O was added to induce phase separation and vortexed and then centrifuged for 5 min at 20,000 × g. Subsequently, 1000 µl of the upper layer was collected into a different tube and evaporated to dryness with a centrifugal evaporator for approximately 3 h at 30 °C. The residue

was dissolved again in 500 µl of mobile phase B used for high-performance liquid chromatography (HPLC) separation (described below). The concentrate was filtrated through a 0.2 µm pore size membrane syringe filter (Minisart RC-4, Sartorius, Germany) and used for LC-MS/MS analysis.

3.3.3 Lipid separation and detection

Lipid analysis was conducted by the liquid chromatography–mass spectrometry system consisted of an HPLC system (Prominence, Shimadzu, Kyoto, Japan) and a linear ion trap triple–quadrupole mass spectrometer (QTRAP 4500, AB-Sciex, Framingham, MA, USA). Briefly, an autosampler injected 10 µl of sample extract into the system. Then, liquid chromatography separation was performed at 40 ℃ using a reverse-phase column (Cadenza CD-C18, 100 × 2.0 mm i.d., 3 µm in particle size, Imtakt, Kyoto, Japan) at a flow rate of 0.35 ml min^{-1}. Next, gradient elution was applied for preliminary separation via HPLC using the mobile phases of 20 mM ammonium acetate, isopropanol, and methanol (7/1/3, v/v/v) containing 0.01% acetic acid for A, and 20 mM ammonium acetate, isopropanol, and acetonitrile (1/7/3, v/v/v) containing 0.01% acetic acid for B. In the gradient program, the ratio of the mobile phase B was started at 40% for 1 min, increased to 80% B in 3 min, and then to 95% B in 4 min, followed by a linear gradient to 100% B in 4 min, maintained at 100% B for 14 min, and then decreased to 40% B and kept constant for 2 min to equilibrate the column for next injection.

The eluent from liquid chromatography was introduced to the mass spectrometer for further mass separation and detection. First, an electrospray ionization using a Turbo-V™ ion source was conducted at 300 °C with −4.5 and 5.0 kV of spray voltage for negative and positive ionization mode, respectively. Other conditions were set as follows: ion source gas 1 (sheath gas), 50 psi, and ion source gas 2 (drying gas), 80 psi. Mass separation and detection were performed using multiple reaction monitoring (MRM) mode, where the transition from

precursor ion (Q1) and product ion (Q3) of each target lipid, were set on the basis of the predicted fragmentation pattern (Tarazona et al. 2015). In this study, 1,347 lipid species were targeted. To obtain enough number of data points in the MRM chromatogram and a high signal-to-noise ratio for accurate quantification, the mass spectrometry analysis was conducted separately in six batches containing approximately 200 transitions per batch. Each transition was performed with a dwell time of 5 and 4 ms for the positive and negative modes, respectively. The MRM transitions and setting parameters, such as the collision energy applied for each species are shown in Table S1–S6.

The mass spectrometry analysis was conducted in five replicates from five different cabbage samples, and two injections were performed for each sample. The sequence of testing sample injection was randomized so as not to be biased by the sensitivity fluctuation of the mass spectrometer. Additionally, the pooled quality control (QC) sample, prepared by mixing a small aliquot of each testing sample, was injected every five samples for monitoring and correcting the drift of the sensitivity of the mass spectrometer.

3.3.4 Structural confirmation of selected lipid species by product ion scanning

For each lipid species selected as an important molecule by the multivariate analysis described later, MRM information-dependent acquisition-enhanced product ion scan (MRM-IDA-EPI) was applied to get more structural information, increasing annotation accuracy. The MRM-IDA-EPI is a kind of auto MS/MS, where the product ion scanning is triggered when the signal intensity exceeds the setting level. In this study, 2,000 cps was set as a triggering criterion, and the product ion spectra were acquired from 50 to 900 Da with dynamic fill time and a scan rate of 10,000 Da s^{-1}. The collision energy to get the fragment was appropriately adjusted depending on the analyte. The values of declustering potential (DP) and entrance potential (EP) were set at 120 and 10 V, respectively. The condition of the ion source was the same as the MRM acquisition mentioned above. Subsequently, the product ion spectra at the

retention time corresponding to the target analyte were compared with the predicted spectra recorded in online databases, such as Human Metabolome Database (www.hmdb.ca) and Lipid Maps (www.lipidmaps.org). The specific *m/z* signals characterizing the target analyte in product ion spectra were annotated to validate structural matching.

3.5 Pre-data processing

The peak picking from the MRM chromatogram and the integration of the peak area were performed using MarkerView software (AB-Sciex, Framingham, MA, USA). The parameter settings for processing were as follows: Gaussian smoothing of five points, a baseline subtraction window of 1 min, noise percentage of 50%, a peak splitting factor of four points, a minimum required intensity of 1,500 cps, a minimum peak width of four points, and a minimal signal/noise of 300. Each peak area in tested samples was normalized by that of internal standards and sample weight after applying the QC-based-robust locally estimated scatterplot smoothing (LOESS) signal correction (QC-RLSC) using pooled QC sample data (Dunn et al., 2011). Normalization and QC-RLSC were conducted using in-house R scripts (Ver. 3.6.2, R Foundation for Statistical Computing).

Statistical analysis was conducted to evaluate the lipidome alteration depending on cumulative CO_2 production. First, a regression analysis was performed to investigate the linearity between the abundance of each detected lipid species and the cumulative CO_2 production. Then, the hypothesis test for the regression coefficient was done using R (Ver 3.6.2, The R Foundation for Statistical Computing) to pick out the lipid species for further multivariate analysis. The screened lipid data were served to hierarchical cluster analysis (HCA) with metric Pearson correlation and Ward linkage, and their profiles were visualized on MetaboAnalyst 5.0 (www.metaboanalyst.ca).

Subsequently, PLSR was applied to elucidate the important lipid species that strongly relate to the increment or decrement of cumulative CO_2 productions using SOLO (Ver.8.9,

Eigenvector Research Inc., Manson, USA). In PLSR, each normalized peak area of lipid species and the cumulative CO_2 productions were set as explanatory (X) and objective variables (Y), respectively. A total of 64 datasets, collected from three levels of storage temperatures for six levels of storage durations in five replications, were used in PLSR splitting into a training (38 datasets) and a test set (26 datasets). Prior to the model development, both explanatory and objective variables were standardized by autoscaling (mean-centering and scaled to unit variance). Venetian blinds cross-validation was applied in building a PLSR model, where the maximum number of latent variables (LVs) and the number of data splits were set as 20 and 10, respectively. Then, the number of LVs was chosen on the basis of the lowest root-mean-square error of calibration (RMSEC). Afterward, the root-mean-square error of cross-validation (RMSECV), R^2Y, and Q^2Y were utilized to evaluate model performance. A permutation test (n = 100) was also conducted to confirm model robustness. Finally, on the basis of the developed model, important lipid species were selected according to both variable importance in projection (VIP) scores and the p-value of the significance test of the correlation coefficient in univariate analysis.

IV. RESULT AND DISCUSSION

4.1 Change of CO_2 production rate of cabbage during storage

Figure 1 presents the changes in CO_2 production rate in cabbage during storage at 5 °C, 10 °C, and 20 °C. The CO_2 production rate was suppressed more when cabbage was stored at lower temperatures. The changes at 5 °C and 10 °C were almost stable throughout the storage period. In the case of storage at 20 °C, the CO_2 production rate decreased drastically in the first 100 h and then became steady. Since the respiration rate strongly correlates with quality degradation, it has been used as a benchmark of the perishability or storability of fresh produce after harvesting in postharvest technology studies. According to Kader (2002), the respiration rate of fresh produce is classified into six levels, and cabbage is categorized in the moderate level of respiration rate. Brash et al. (1995) revealed that the cumulative respiratory CO_2 production after harvest of asparagus stored at various temperatures had a strong negative correlation with residual shelf-life at 20 °C and is suggested to be the same in other crops. The loss of ascorbic acid in broccoli, which is an important quality attribute, could also be predicted by the model as a function of the cumulative reparatory CO_2 production after harvest (Techavuthiporn et al. 2008). On this basis, Syukri et al. (2018) utilized the cumulative CO_2 production as a reference of the freshness degree and succeeded in identifying the freshness maker of soybean sprouts by the comprehensive analysis of carbonyl compounds using LC-MS/MS. Li et al. (2021) also demonstrated the capability of visible and near-infrared (Vis-NIR) spectroscopy in estimating the freshness of Japanese mustard spinach by using it as a reference of freshness. Thus, cumulative CO_2 production from the beginning of the storage to sample collection was applied as a reference of the freshness degree of cabbage in later analysis and discussion in the present study.

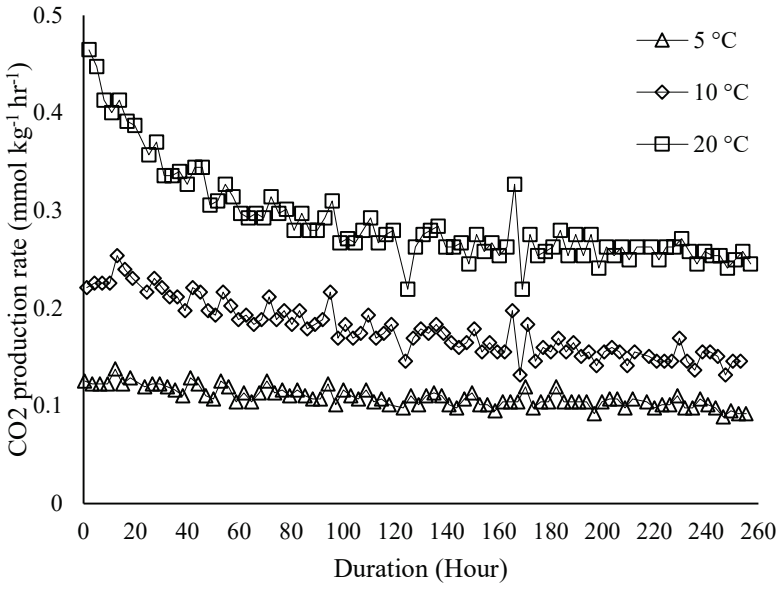


Fig. 3. Changes in the CO_2 production rate of stored cabbage at various temperature conditions.

4.2 Lipid profile in cabbage stored at different cumulative CO_2 productions

Lipids in cabbage samples stored at different temperatures and durations were comprehensively analyzed using LC-MS/MS. In total, approximately 600 peaks were detected. Only signals having less than 20% relative standard deviation (RSD) in peak area reproducibility across the QC sample were screened. Approximately 170 peaks fulfilled the above criteria and were then subjected to simple regression analysis to examine the linearity against the cumulative CO_2 production. According to the regression coefficient, a total of 74 lipid species including 13 species of PC, 17 species of PE, three species phosphatidylglycerol (PG), three species of phosphatidylinositol (PI), two species of lysoglycerophospholipid (LGPL), seven species of monogalactosyldiacylglycerol (MGDG), three species of digalactosyldiacylglycerol (DGDG), eight species of sulfoquinovosyldiacylglycerol (SQDG), three species of sphingomyelin (SPM), three species of acylated steryl glycosides (ASG), three species of diacylglycerols (DAG), and nine species of triacylglycerols (TAG) were significant. Significantly different species were subsequently analyzed further via multivariate analysis.

The profiles of screened lipid species were visualized as a heatmap and a dendrogram obtained by HCA shown in Fig. 4. According to the HCA, lipid species are clustered into three main groups. The first group had an upward trend, and the second and the third groups had a downward trend. Furthermore, the decreasing rate of the second group was faster than that of the third one.

The first group comprised the lipid molecules belonging to TAG and ASG classes except for DAG 18:3_18:3. TAG is well known as a storage lipid and coalesces into lipid droplets in the cytoplasm. Generally, TAG maintains homeostasis in the cell membrane. Considering the lipid metabolism pathway, the increment of TAG observed in this study was implied that DAG and/or other phospholipids and glycolipids converted into TAG during senescence. An increase in TAG was also observed in Arabidopsis leaves during the aging process. Research suggests that it is caused by the detachment of FAs from glyceroglycolipid in the thylakoid membrane followed by TAG formation in plastoglobuli (Kaup et al. 2002; Watanabe et al. 2013). Mueller et al. (2017) revealed that TAG accumulation occurred when Arabidopsis leaves were exposed to heat stress; also, they suggested that the accumulation of TAG could be a way to improve heat tolerance in plants. By contrast, Lin and Oliver (2008) mentioned that the conversion of galactolipids to TAG caused metabolic disruption in crabapple leaves during senescence.

Sterols are natural organic compounds that have an essential function in all eukaryote cell membranes. They are also a membrane reinforcer in lipid rafts that contribute to maintaining membrane fluidity and permeability. Sterols interact with sphingolipids to influence membrane properties to aid in adapting to environmental changes (Dufourc 2008). Sterols divide into free sterols, namely, steryl esters (SEs), steryl glycosides (SGs), and acyl steryl glycosides (ASGs). SGs and ASGs are secondary metabolites that are widely distributed in plants. SG is a hydroxyl group of C3 from sterol bound to sugar, synthesized by sterol glucosyltransferase (SGT). The sugar moiety from SGs is acylated with FA in the C6 hydroxyl group to form ASGs (Ferrer et

al. 2017). In this study, several ASGs were detected in stored cabbage and increased with cumulative CO_2 production. Li et al. (2016) also reported that ASGs increased, but SEs decreased during senescence in tobacco leaves. Takahashi et al. (2016) observed changes in ASGs during cold acclimation in two types of plants: ASG content increased from 68.2% to 71.7% in oat, whereas it decreased from 14.7% to 7.7% in rye. Although different behavior was observed between them, these facts implied that cold acclimation and senescence were caused by alterations in physiological functions of the microdomains in the cell membrane.

The molecular species in the second group were mainly from the glyceroglycolipid class such as MGDG, DGDG, and SQDG. The third group was dominated by species from the glycerophospholipid class such as PC and PE. These classes are abundant in plants, mainly located in the biomembrane. MGDG, DGDG, and PC decreased during senescence in barley leaves (Wanner et al. 1991). Decreasing of MGDG was caused by the conversion to DAG by galactolipid–galactolipid galactosyltransferase, followed by the conversion to PC. Then, PC enters the glycolysis pathway or it is hydrolyzed to PA (Wanner et al. 1991; Jia et al. 2013). This journey is almost in accordance with Li et al. (2016), showing MGDG, DGDG, SQDG, and several lipids in the PC and PE classes decreased in the early senescence of tobacco leaves. Watanabe et al. (2013) also stated that decreasing MGDG, DGDG, SQDG, and PG in thylakoid occurred during senescence in rosette leaves, and their alteration correlated with the degradation of chlorophyll.

Interestingly, in this work, the rate of decline in MGDG, DGDG, and SQDG was faster than in PC and PE classes. Previous research showed that phospholipase activity such as phospholipase D (PLD), PA phosphatase, and lipolytic acyl-hydrolase trigger the breakdown of membrane phospholipids (Brown et al. 1987). Particularly, Jia et al. (2013) mentioned that an increase of PLDδ activity stimulated the degradation of PC, MGDG, and DGDG during ABA-promoted senescence in Arabidopsis. MGDG and DGDG decreased more than PC under

the increase in PLDδ activity. The plastidic lipids such as MGDG, DGDG, and SQDG degraded rapidly during ethylene-promoted senescence when compared with extraplastidic lipids such as PC, PE, and PI (Jia and Li 2015).

To date, lipid class-based analysis has been mainly conducted to investigate the behavior of lipid alteration in stress response. However, as shown in Fig. 4, a molecular-based analysis revealed that lipid molecules belonging to the same class grouped into different clusters. For example, DAG 18:3_18:3 had an upward trend (group 1), whereas DAG 18:0_18:3 and 18:1_18:3 had a downward trend (group 3). These results indicated that even in the same class of lipids, the hydrophobic tail has a significant impact on the stress response. However, there are few studies examining stress response and aging of plants at the lipid molecular level. The findings presented here by the lipidomics approach will provide useful information to elucidate the mechanism of lipid dynamics associated with plant senescence.

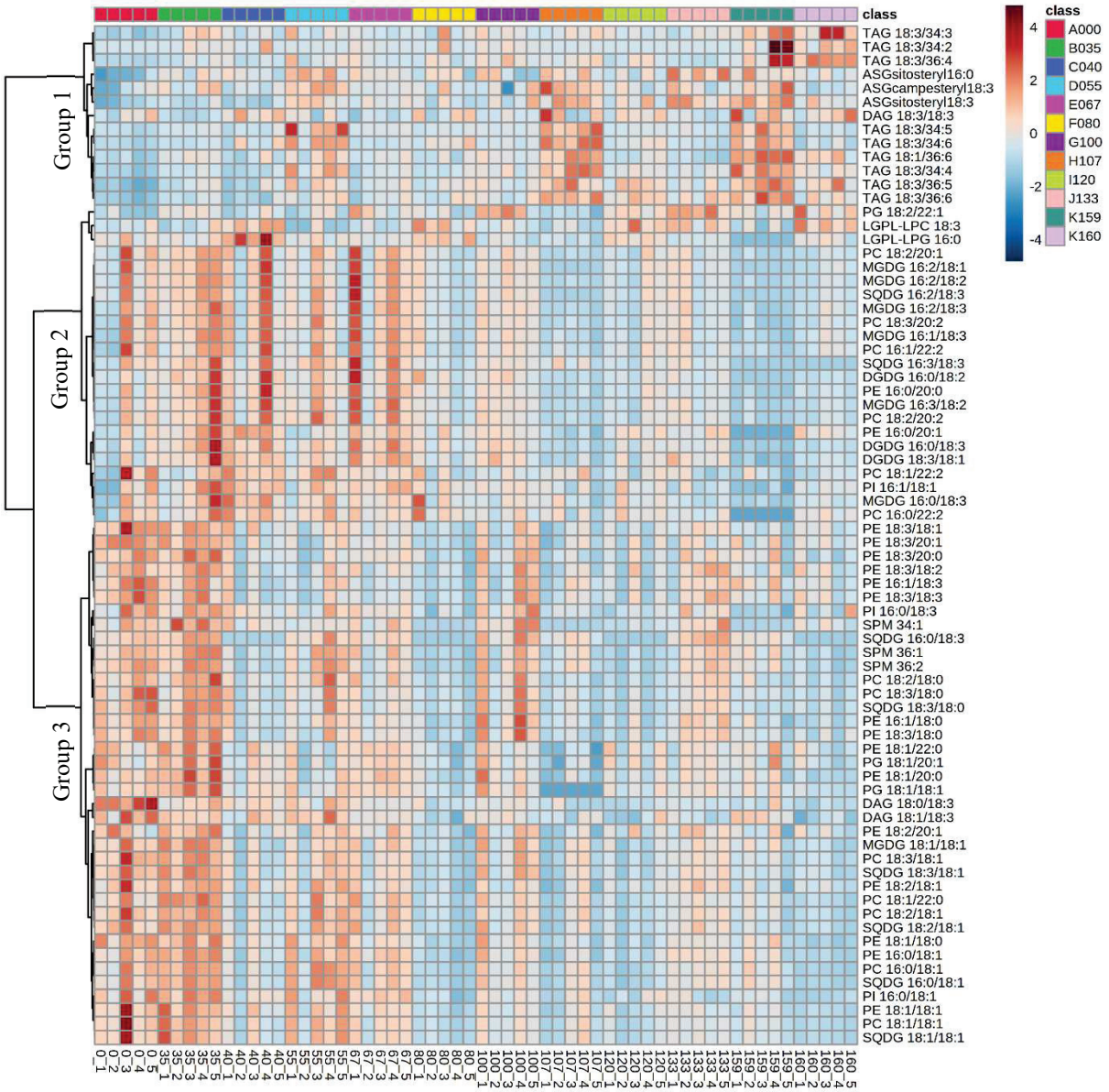


Fig. 4 Heatmap of average autoscaled lipid abundance changes in stored cabbage with the progress of the cumulative CO_2 production; 74 significant species are presented ($p < 0.05$ in the slop test of the simple regression analysis). The different colors at the top of the heatmap show the level of cumulative CO_2 production. A000 to K160 indicate the cumulative CO_2 production from 0 to 160 mmol kg^{-1}.

4.3 PLSR analysis of lipids in cabbage during storage at different temperatures

PLSR is a multivariate machine learning algorithm that allows datasets with more variables than samples to be modeled without resorting to prefiltering variables. Moreover, once optimized, a PLS model can be reduced to the form of a standard linear regression, from which inference about the importance of variables can be made (Mendez et al. 2019). For these reasons, PLSR has become the gold standard in metabolomics where a large member of metabolites interacting with each other in a biological system is simultaneously analyzed. It has been used for metabolic fingerprinting, profiling (Gao et al. 2020), and screening of biomarkers (Zhou et al. 2021). We performed PLSR to determine the relative response of 74 selected species and cumulative CO_2 production as explanatory (X) and predictor (Y) variables, respectively. As a result of the model development by internal cross-validation, the model with four LVs had the minimum RMSECV. Figure 5 shows the scatter plot of measured versus predicted cumulative CO_2 production using the developed model. The fraction of the sum of the square of all the Y's explained by the PLS model (R^2Y) and that of the total variation of the Y's that can be predicted by the PLS model (Q^2Y) were 0.94 and 0.84, respectively. These results demonstrate the strong explanation and prediction capabilities of the model. The permutation test showed that the unpermuted model was significantly different ($p < 0.05$) from the model created with randomly permuted samples. These results indicate that the developed PLS model was not overfitted and was reliable for predicting the cumulative CO_2 production using lipidome data. Additionally, the determination coefficient (R^2) in the external validation using the test dataset, which was not used for the model development, was 0.88. In other words, the fact that a highly accurate PLS model could be created successfully implies the existence of the specific lipid molecule that expresses the freshness of cabbage.

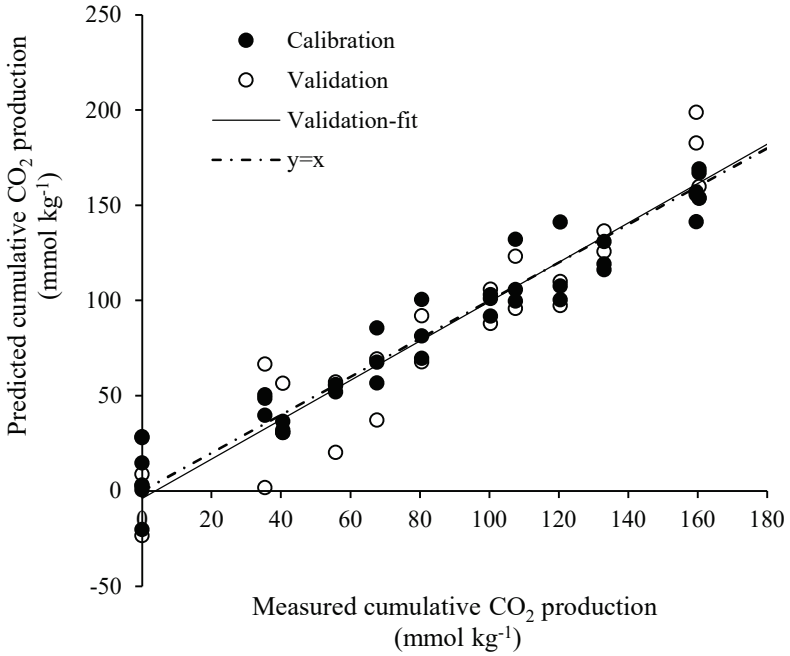


Fig. 5 PLSR plot for predicting the cumulative CO_2 production using 74 species with four latent variables.

Since the VIP scores can rank variables in terms of their importance in the projection of the PLS model, it has often been used to elucidate the potential markers in metabolomics studies (Li et al., 2020; Parijadi, Putri, Ridwani, Dwivany, & Fukusaki, 2018). Thus, we used the VIP score for marker selection in this study. Additionally, to achieve a more reliable marker selection, the p-value of a hypothesis test for the regression coefficient in simple linear regression was considered. The potential freshness markers were selected based on the criteria both >1.4 in VIP score and ≥7 in $-\log_{10}$ (p-value). As a result, four lipid molecules were selected as indicated in Fig. 6.

All selected lipids were validated their annotation by matching the MS/MS spectrum obtained by post-hoc MRM-IDA-EPI scans with putative fragment daughter ions of each lipid molecule (Fig. 7). Particularly, the annotation of TAGs, which have three acyl chains, is impossible in MRM because it detects a target analyte on the basis of the combination of one parent ion (Q1) and one daughter ion (Q3), so it cannot cover the information on the third acyl chain in TAG. However, due to the post-hoc MRM-IDA-EPI scanning, TAG 18:3/36:6 could

be annotated as TAG 18:3/18:3/18/3 because only daughter ions resulting from linolenic acyl chain (18:3) was found in the MS/MS spectrum (Fig. 7 (A)). In the case of TAG 18:3/36:5, because only the signals resulting from linolenic (18:3) and linoleic (18:2) acyl chains were found, it was annotated as TAG 18:3/18:2/18/3 (Fig. 7 (B)). In the case of DAG 18:0/18:3 and ASG-sitosterol 18:3, specific daughter ions characterizing those compounds could be observed in each MS/MS spectrum as shown in Fig. 7 (C) and (D).

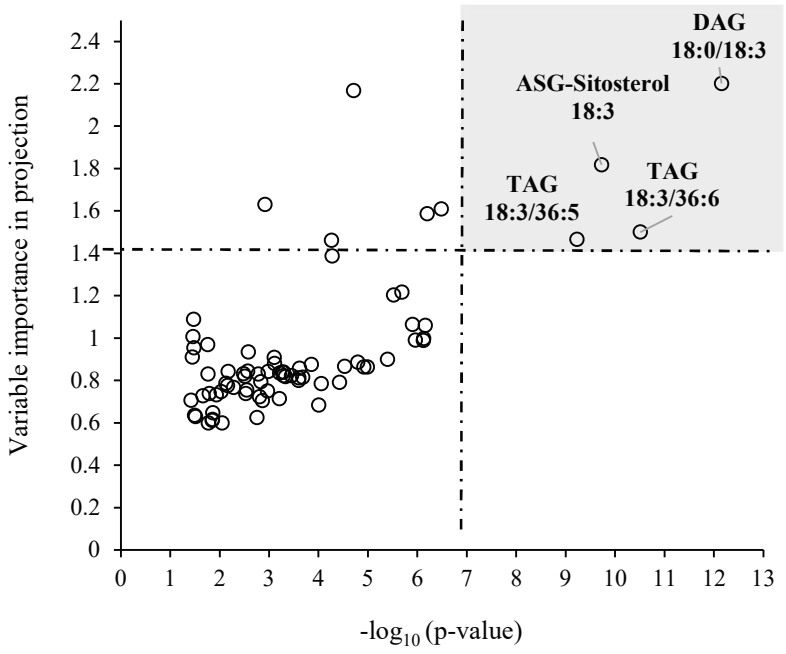
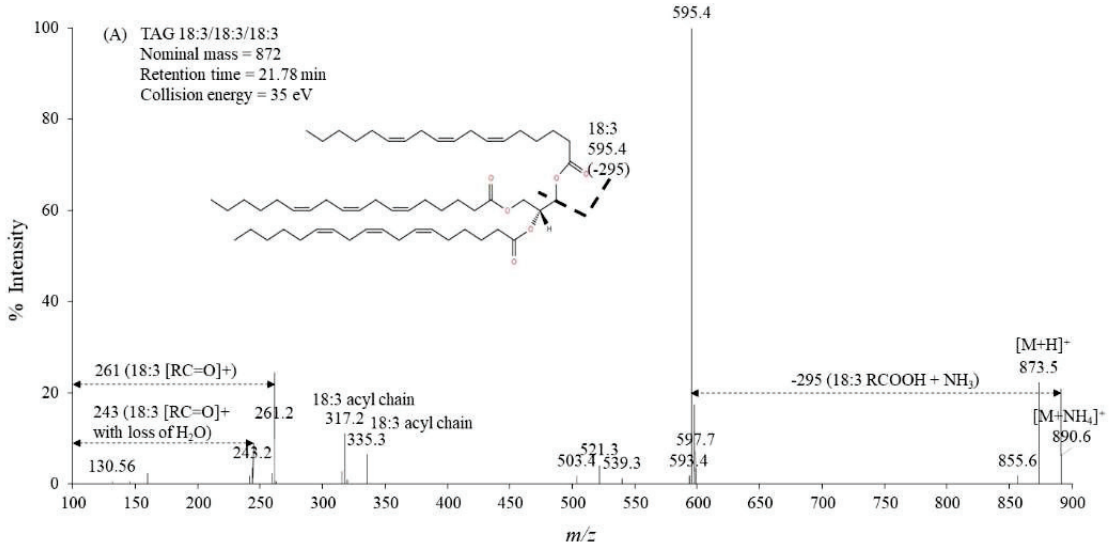
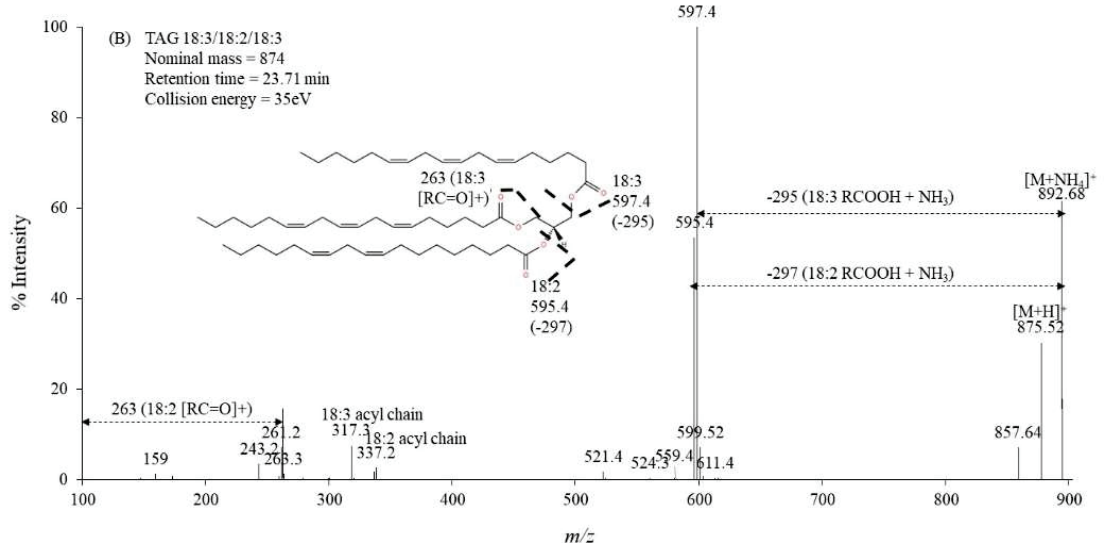
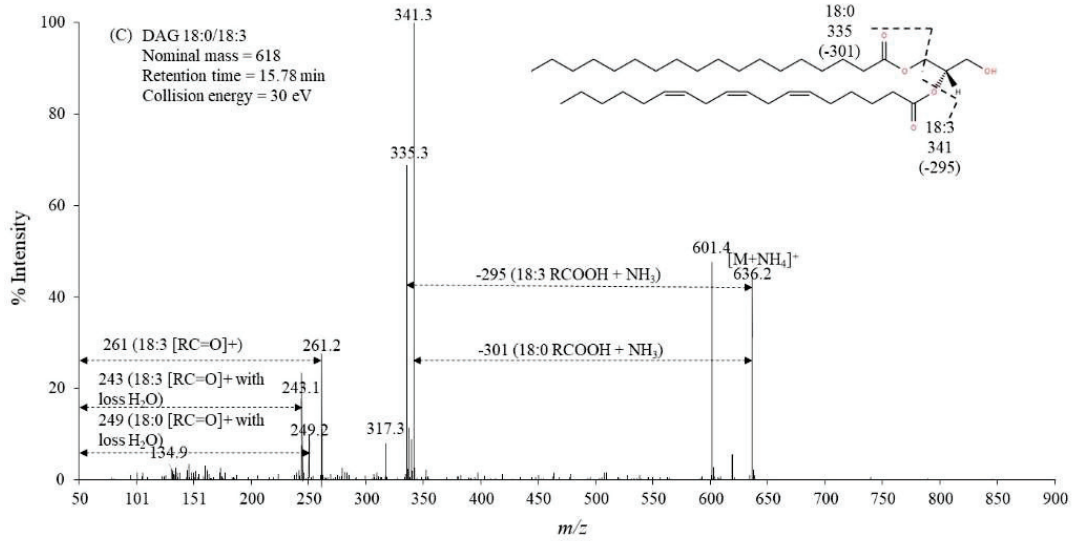


Fig. 6 Selection of the potential freshness marker based on the VIP scores and p-value of the test for correlation coefficient in a simple regression.


(A) TAG 18:3/18:3/18:3
Nominal mass = 872
Retention time = 21.78 min
Collision energy = 35 eV
595.4
18:3
595.4
(-295)
% Intensity
261 (18:3 [RC=O]+)
243 (18:3 [RC=O]+ with loss of H2O)
261.2
243.2
130.56
18:3 acyl chain
317.2
18:3 acyl chain
335.3
503.4
521.3
539.3
593.4
597.7
-295 (18:3 RCOOH + NH3)
[M+H]+
873.5
[M+NH4]+
890.6
855.6
m/z


(B) TAG 18:3/18:2/18:3
Nominal mass = 874
Retention time = 23.71 min
Collision energy = 35eV
597.4
263 (18:3 [RC=O]+)
18:3
597.4
(-295)
18:2
595.4
(-297)
% Intensity
-295 (18:3 RCOOH + NH3)
595.4
-297 (18:2 RCOOH + NH3)
[M+NH4]+
892.68
[M+H]+
875.52
263 (18:2 [RC=O]+)
261.2
243.2
268.3
159
18:3 acyl chain
317.3
18:2 acyl chain
337.2
521.4
524.3
559.4
599.52
611.4
857.64
m/z


(C) DAG 18:0/18:3
Nominal mass = 618
Retention time = 15.78 min
Collision energy = 30 eV
341.3
18:0
335
(-301)
18:3
341
(-295)
335.3
% Intensity
601.4
[M+NH4]+
636.2
-295 (18:3 RCOOH + NH3)
-301 (18:0 RCOOH + NH3)
261 (18:3 [RC=O]+)
261.2
243 (18:3 [RC=O]+ with loss H2O)
243.1
249 (18:0 [RC=O]+ with loss H2O)
249.2
317.3
134.9
m/z

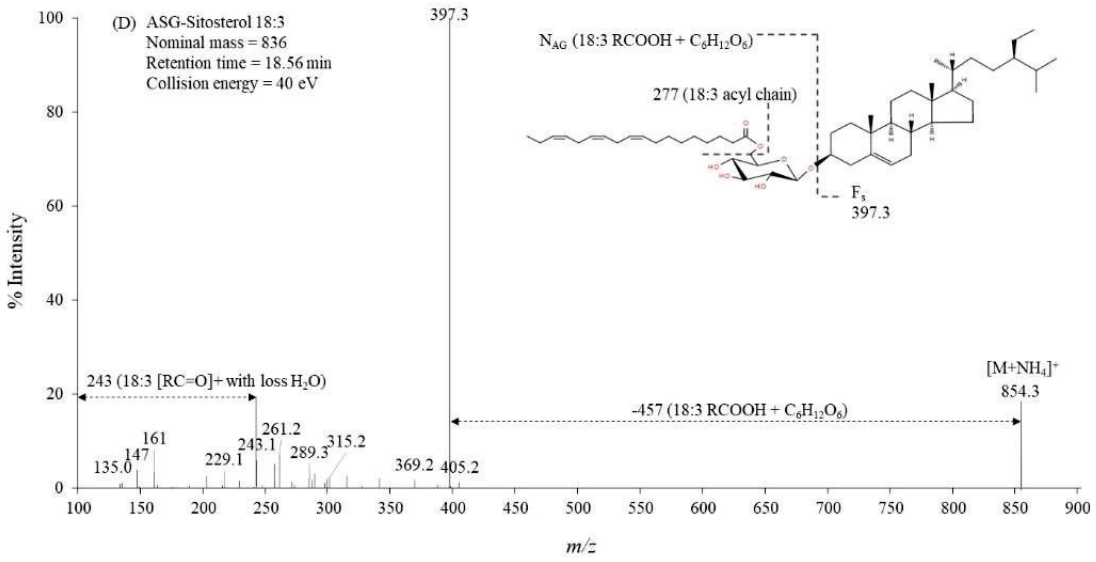


Fig. 7 Confirmation of potential freshness markers by product ion spectra

4.4 Performance of Potential Freshness Markers in Predicting Cumulative CO_2 Production

In this study, we observed the alteration trend of lipid species to evaluate the freshness in whole cabbage. DAG 18:0_18:3, ASG-sitosterol 18:3, TAG 18:3_18:3_18:3, and TAG 18:3_18:2_18:3 were selected to be potential freshness markers in stored cabbage. The coefficient determinant (R^2) of the relationship between the cumulative CO_2 production during storage and each potential freshness marker ranged from 0.46 to 0.57 (Fig. 8). Based on the R^2 values, it is difficult to accurately evaluate the cabbage freshness by using a single marker. Hence, the PLSR model was rebuilt using the four selected potential markers, and its performance was evaluated. Figure 9 shows the measured and predicted cumulative CO_2 production by the PLSR model using four potential markers with two latent valuables. R^2Y and Q^2Y values were 0.74 and 0.71, respectively, indicating moderate predictability and high robustness of the model. The root-mean-square error of prediction (RMSEP) of this model was 29.9 mg CO_2 kg^{-1}, which corresponded with 10, 6, and 3.5 days of storage at 5 °C, 10 °C, and 20 °C, respectively. The determinant coefficient of prediction (R^2_{pred}) was 0.69. Considering

that cabbage is stored at low temperature for a maximum of 4 months in the fresh-cut vegetable industry, this level of model error is considered to be acceptable in practical use.

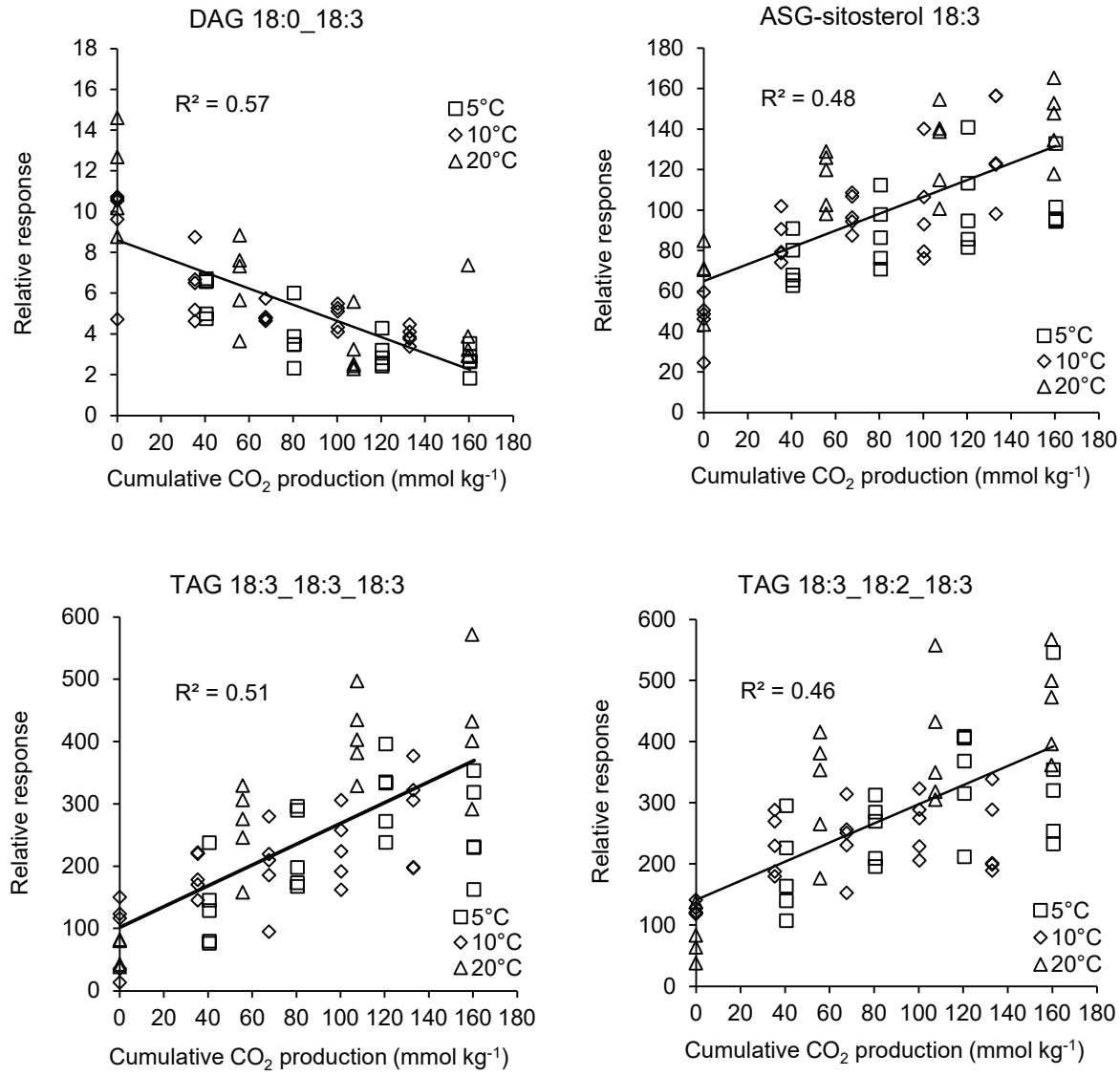


Fig. 8 Relationship between the relative response of potential marker and cumulative CO_2 production of stored cabbage

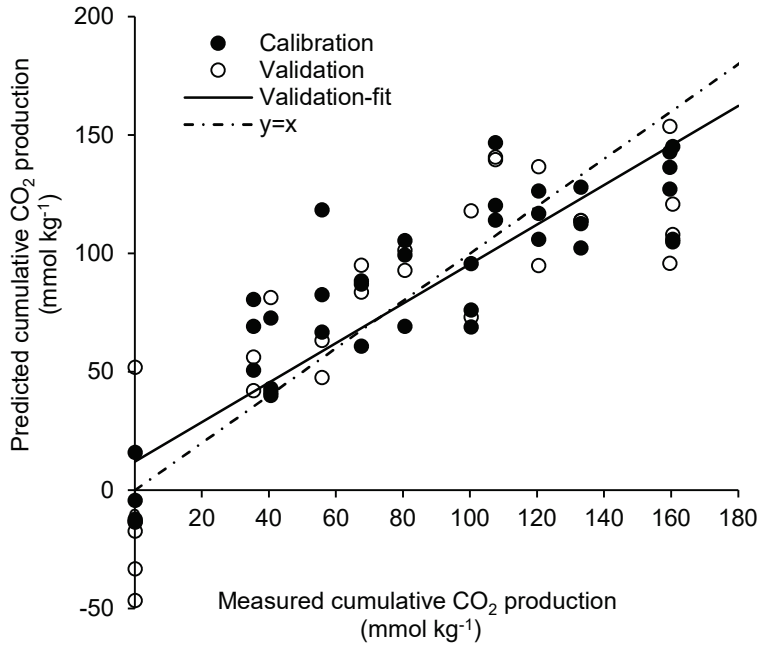
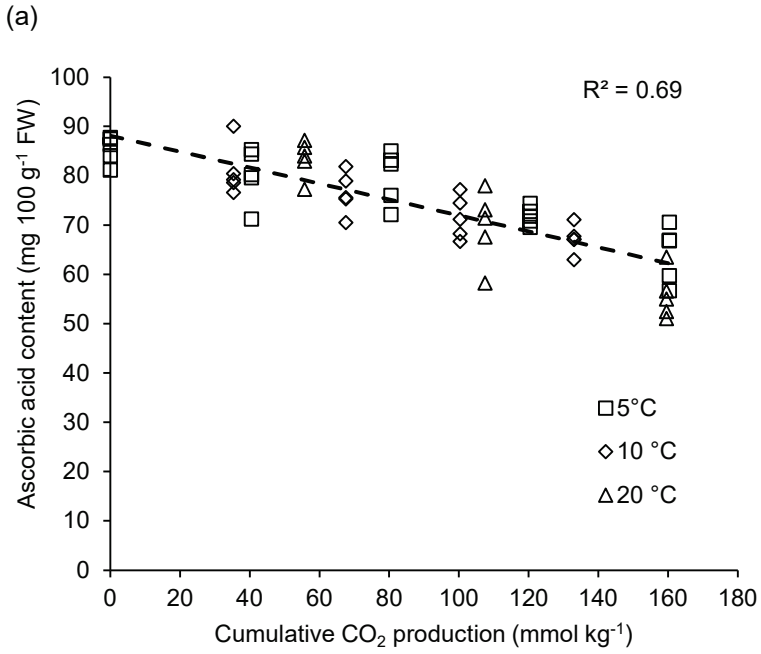


Fig. 9 PLSR plot for predicting cumulative CO_2 production. The PLSR plot was developed using four potential markers with two latent variables

AsA is one of the major quality attributes of fruits and vegetables after harvesting. Since AsA declines depending on the storage duration and temperature, it has been employed to quantify the quality degradation of fruits and vegetables during storage (Saito et al. 2000; Yamauchi and Kusabe 2001; del Aguila et al. 2006; Galani et al. 2017). Hence, we compared the performance of selected four lipids and AsA in predicting the cumulative CO_2 production. AsA in stored cabbage decreased with an increase of the cumulative CO_2 production as shown in Fig. 10a. Using the same method describe earlier, PLSR was performed to build the predictive model using AsA as an explanatory value. Figure 10b shows the relationship between measured and predicted cumulative CO_2 production using the developed PLSR model by AsA. The RMSEP and R^2_{pred} was 33.0 mg CO_2 kg^{-1} and 0.58, respectively. This result indicates that the model with four important lipid molecules can express the degree of freshness of cabbage more accurately than AsA which has been used in freshness assessment conventionally.

In this study, we succeeded in identifying four important lipid molecules that express the degree of freshness of cabbage. To verify the effectiveness of freshness assessment by lipidomic profiling, tests using other kinds of vegetables and considering stress factors other than temperature that fresh produce endure during distribution such as gas modification, vibration, and impact should be conducted in future research.



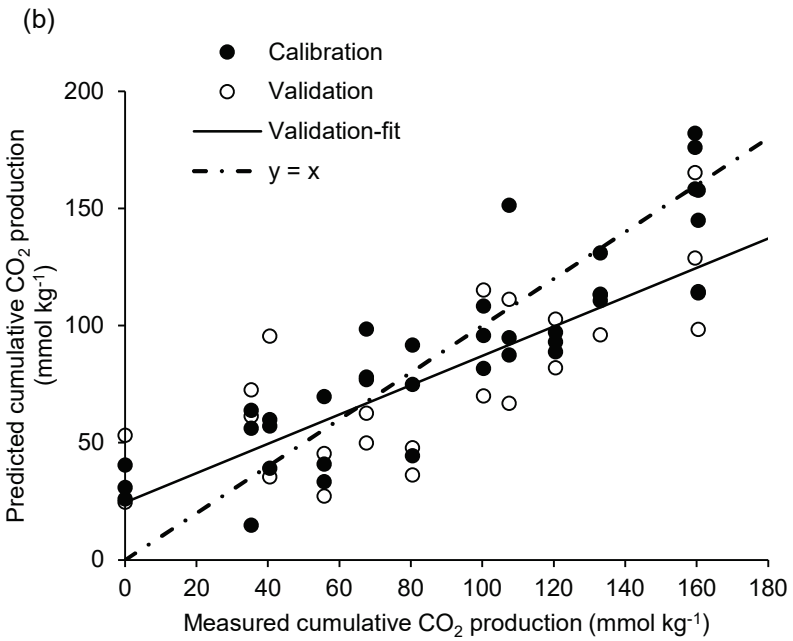


Fig. 10 The changes of AsA in stored cabbage with an increasing of cumulative CO_2 production **(**a), and the performance of PLSR model using AsA for predicting the cumulative CO2 production (b)

V. CONCLUSION

Agricultural products such as fruits, vegetables, legume, species, and nuts are important in developing civilization and economies because it has become the complementary nutritional for the life span of a human. The nutritional compound is essential for human health, such as diets and the prevention of chronic diseases. Due to this benefit, agricultural products, especially fruits and vegetables, have become popular among consumers. Despite this popularity, fruits and vegetables have unique phenomena after harvesting. This fresh produce continues its metabolic activity, affecting nutrition losses and short shelf life.

Recently, the term freshness is most famous on the consumer, and it is a common word encountered in daily life. Freshness can describe the quality of fresh produce that have optimal conditions for consumer acceptance. Because freshness represents all quality attribute such as appearance, flavor, nutritional compounds, and phytochemical. In practical, freshness evaluation often using the conventional method that measures the specific quality attribute. This is the great challenge for researcher to develop the new method that can improve the common method.

In this dissertation, we investigated the lipidome alteration in whole cabbage during storage to identify the freshness markers using liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based lipidomics. Lipidomics is a subset of metabolomics focusing on lipids. Lipids are essential metabolites that have crucial cellular functions. Focusing on lipid can provide the information about cellular metabolic status. It helps understand the biological mechanism and find out the biomarkers based on the comprehensive analysis of lipids.

The whole cabbage was selected as a sample material because it is one of the common vegetables in the world, and the freshness is an important attribute due to its short shelf-life. Moreover, the quantitative freshness assessment method is strongly desired, especially in the fresh-cut fruits and vegetable industry. The raw material cabbage is often stored at low

temperature for a long period, and its freshness is affected to the quality of the final fresh-cut product. For these reasons, the whole cabbage stored at different temperatures and durations was used/ to discuss the feasibility of the freshness assessment by lipidomic profiling in this study.

Wide target lipidomics using a triple–quadrupole mass spectrometer were conducted to understand changes in the lipid profile alteration of cabbage during storage and to find out the specific lipid species that indicate the degree of freshness. Among a large number of detected lipid species, 74 molecules were found to have a significant correlation with the cumulative respiratory CO_2 production during storage. The transition of lipid profile with senescence could be visualized. Moreover, the PLSR model as a function of these lipid molecules could predict cumulative respiratory CO_2 production accurately. Based on the VIP score in PLSR and the p-value of the test for correlation coefficient in a simple regression, we succeeded in elucidating the four potential freshness markers that express the degree of freshness of cabbage. Moreover, the prediction model with these potential markers gave a more accurate result than conventional AsA method in freshness assessment. To the best of our knowledge, this is the first attempt using the lipidomics approach for developing the freshness assessment method of vegetables. To the best of our knowledge, this is the first attempt using the lipidomics approach for developing the freshness assessment method of vegetables.

Additionally, we could clarify the feasibility of the freshness evaluation of cabbage by lipidomic profiling. Further research is needed to verify the availability of selected potential markers by testing the effect of not only temperature but also other stress factors such as controlled atmosphere and vibration/impact. Furthermore, the quantitative analysis of the selected potential freshness markers should be undertaken to develop a robust freshness measurement model. Then, it should be combined with non-destructive technology for the

practical use of freshness assessment. Nevertheless, we believe that these preliminary findings will be a primary reference in postharvest science to explore freshness assessment further.

PUBLICATIONS

Zainal, P. W, Aurum, F. S, Imaizumi, T., Thammawong, M., Nakano, K. 2022. Application of mass spectrometry-based metabolomics in postharvest research. Reviews in Agricultural Sciences. 10, 56-67.

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