

Applicability of a Respiration-Based Ascorbic Acid Prediction Model to a Range of Selected Vegetables

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Abstract

To confirm the applicability of the respiration-based total ascorbic acid (AA ; L-ascorbic and dehydroascorbic acids) prediction model, described in our previous research, to a wide range of commodities, the relationship between AA change and the CO₂ production rate in stored cabbage, cauliflower, spinach and green pepper was investigated in an experiment involving various temperature conditions (5, 10, 20 and 30°C). The percentage of relative AA (AA_{rel}), which was normalized against the initial value, was plotted against the accumulated amount of CO₂ production (AR_{CO_2}). AA_{rel} decreased with increasing AR_{CO_2} in cabbage, cauliflower and spinach. The relationship between AA_{rel} and AR_{CO_2} was well expressed by the equation ($AA_{rel} = 100 \exp(-\beta AR_{CO_2})$) in these products. On the other hand, this relationship could not be observed in green pepper (*Capsicum*), which was considered a limitation of the model. The statistical test for homogeneity of regression coefficients showed that there was no difference among the β parameters of broccoli (previous data), cabbage and cauliflower, all of which belong to the genus *Brassica*. The β parameter of spinach, belonging to the genus *Spinacia*, was significantly different from the other commodities. The results obtained in this study showed that our proposed model is applicable to not only for broccoli but also for other commodities. The model could be a useful tool in designing the optimum distribution chain for these products.

[Keywords] ascorbic acid, respiration rate, modelling, *Brassica*, *Spinacia*, *Capsicum*

I Introduction

Supplying high quality products to consumers and reducing postharvest losses through the distribution chain are the primary objectives in the field of postharvest technology. Quality prediction is one of the prospective approaches towards fulfilling these objectives. Therefore, several attempts have been made in recent years to express quality-related characteristics using mathematical equations (Tijskens et al., 1998 ; Nourian et al., 2003 ; Piagentini et al., 2005 ; Villanueva et al., 2005). Most of these proposed models are based on the theory of chemical reaction kinetics ; some of them are described as a function of time and temperature. However, since the field of quality modelling is relatively new, the information available is limited for a wide range of commodities.

On the other hand, the respiration modelling appears to be more advanced than quality modelling with regard to model variation and the completeness of parameter data. Until date, the bulk of information on respiration modelling has been collected for various

types of fresh produce. This is because the respiratory mechanism is at the root of deterioration of such produce and reflects their physiological state (Cameron et al., 1989 ; Lee et al., 1991 ; Beaudry et al., 1992 ; Hagger et al., 1992 ; Peppelenbos and van't Leven, 1996 ; Peppelenbos et al., 1996 ; Talasila and Cameron, 1997 ; Hertog et al., 1998 ; Jacxsens et al., 2000 ; Fonseca et al., 2002 ; Iqbal et al., 2005). These respiration models are currently widely used to determine optimum distribution and storage conditions. In particular, they aid in the development of modified atmosphere packaging systems. If the respiration characteristics were correlated quantitatively to the change in quality, then quality prediction would be possible for a wide range of commodities.

To obtain extensive data from the respiration models used for quality prediction, we investigated the relationship between the respiration characteristics of broccoli and its total ascorbic acid (AA ; L-ascorbic and dehydroascorbic acids) content, which is an important quality indexes for fresh produce. We thereby succeeded in developing a simple empirical

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equation describing the AA content change as a function of respiration. Under the conditions of fluctuating temperature and gas concentrations occurring in the distribution chain, the degradation of the AA content in broccoli could be successfully calculated by substituting the respiration data and the models from the previous studies into our simple AA equation (Techavuthiporn et al., 2008). This confirmed the novel application of the accumulated knowledge on respiration for characterizing AA behaviour in broccoli. The next step in our studies was to determine the applicability of our model to not only broccoli but also other commodities. If a similar quantitative relationship can be established for a wide range of products, our findings would contribute to expanding the field of quality modelling. The results obtained will be useful in determining and managing the conditions under which fresh produce is stored and distributed.

In this study, we investigated the relationship between the AA content and respiration changes in broccoli (*Brassica oleracea* L.), cabbage (*B. oleracea* Capitata group), cauliflower (*B. oleracea* Botrytis group), spinach (*Spinacia oleracea* L.) and green pepper (*Capsicum annuum* L.). These vegetables were selected because they exhibit a wide species variation and respond differently to storage conditions. Our AA equation was fitted to the data of each vegetable, and a model parameter was estimated. The difference between the model parameter values of each selected vegetable was then evaluated statistically. Finally, the applicability of our empirical AA model was discussed.

II Materials and methods

1. Preparation of materials and storage conditions

Broccoli, green cabbage, cauliflower, spinach and green pepper were used in this study. They were obtained from a wholesaler in Gifu City, Japan, and were transported to the laboratory. All products were first sorted for uniformity and appearance. The green cabbage was then cut into pieces (about 10×15 cm), the cauliflower heads were divided into individual florets, and the green pepper was cut into 8 slices lengthwise.

The samples were stored in acrylic containers (50×30×30 cm) placed in incubators (CR-41 ; Hitachi Co., Japan) at 5, 10, 20 and 30°C, respectively ; the humidity level in containers was increased using trays of water (87–97% RH). Data for broccoli was obtained from a previous study (Techavuthiporn et al., 2008).

2. Respiration rate measurement

The respiration rate of the samples was measured using the closed system method. Each sample was weighed (about 300 g of cabbage and cauliflower, and about 200 g of spinach and green pepper) and placed in

a 3l gas-tight glass desiccators inside an incubator at 5, 10, 20 and 30°C, respectively. Using a gas-tight syringe, a gas samples (0.2 ml) from the desiccators were withdrawn from the desiccators through a septum, which was fitted in the desiccators. The samples were then injected into a gas chromatograph (GC-14B ; Shimadzu Co., Japan). A thermal conductivity detector was used for analyzing the gas samples. A Pora-Pack-Q column was used to separate CO₂. Headspace gas was sampled three times in 15-min intervals. The respiration rate in CO₂ production was calculated from the linear regression curve of CO₂ concentration increase. The slope of the regression line was multiplied by the free volume and then divided by the sample weight to obtain the respiration rate in mg CO₂/kg/h (Kang and Lee, 1997). The CO₂ production rate was then converted to the accumulated amount of CO₂ production (AR_{CO_2} ; mg CO₂/kg) by integrating the respiration over the storage time period.

After each measurement, the sample was returned to the storage chamber until further measurement.

3. Ascorbic acid analysis

For the ascorbic acid analysis, we used samples different from those used for the respiration rate measurement were used. Parts of each sample (about 30 g) were cut and blended using a food processor to obtain homogeneous samples. Two grams of the homogenate was ground with 8 ml of 5% metaphosphoric acid solution (Kishida Kagaku Inc., Japan) using a mortar and pestle, and was centrifuged at 15,000×g for 15 min. One milliliter of the supernatant was analyzed for ascorbic acid by the method of Roe et al. (1948). All the L-ascorbic acid content in the extraction was transformed to dehydroascorbic acid by adding 0.5 ml of 0.2% 2,6-dichloroindophenol sodium salt dehydrate (Nacalai Tesque Inc., Japan). One milliliter of 0.5% thiourea solution (Kishida Kagaku) and 0.5 ml of 2% 2,4-dinitrophenol hydrazine (Nacalai Tesque) were added into the testing tubes and incubated at 37°C for 6 hours for hydrolysis. Next, 2.5 ml of 85% sulfuric acid solution (Nacalai Tesque) was added, and then the absorbance of the solution was measured using a spectrophotometer (UV-1600 ; Shimadzu), at 540 nm, from which the AA content was computed. The standard curve was obtained by plotting absorbance against concentration using standard AA solutions (Nacalai Tesque). Thereafter, the total AA (L-ascorbic and dehydroascorbic acids) content was converted to relative AA (AA_{rel}), which was expressed as the remaining content in percentage against the initial value.

4. Relationship between AA change and respiration

Previously, the different simple algebraic equations (linear, exponential and quadratic equation) were tested to describe the relationship between the AA

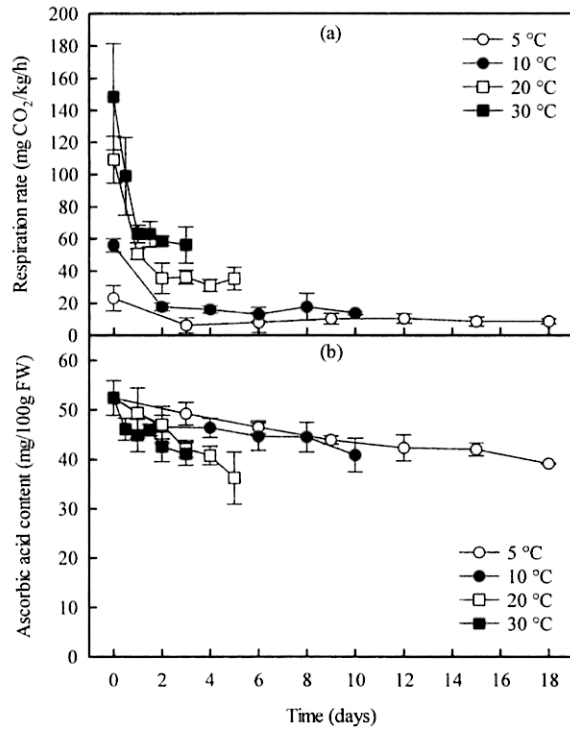


Fig. 1 Changes in respiration rate (a) and ascorbic acid content (b) of stored cut cabbage at various temperature conditions. Each vertical bar represents the standard deviation ($n=3$).

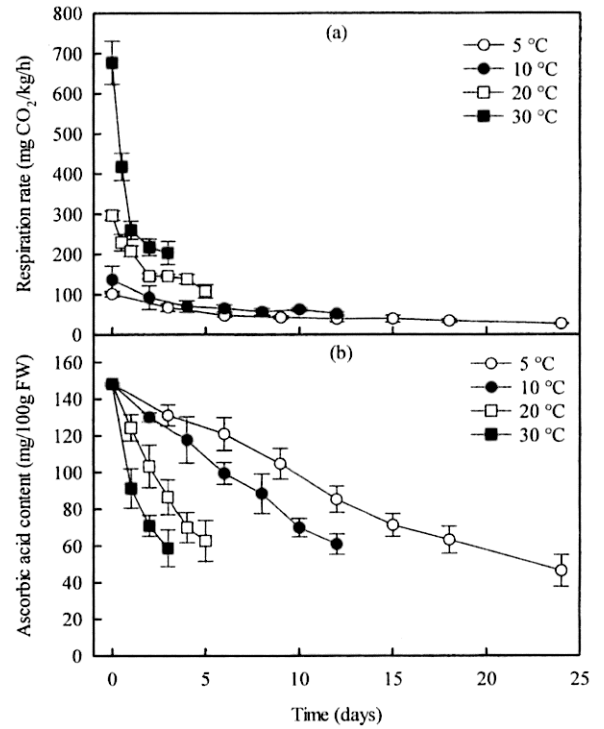


Fig. 2 Changes in respiration rate (a) and ascorbic acid content (b) of stored cut cauliflower at various temperature conditions. Each vertical bar represents the standard deviation ($n=3$).

and respiration changes. The exponential equation was shown to appropriately characterize the AA change in broccoli (Techavuthiporn et al., 2008). Therefore, we expressed the relationship between the decreasing the AA content and increasing AR_{CO_2} is expressed as

$$AA_t = AA_0 \exp(-\beta AR_{CO_2}) \quad (1)$$

where AA_t and AA_0 are the AA content at time t and the initial value (mg/100gFW), respectively; β is the model parameter (kg/mg CO_2); and AR_{CO_2} is the accumulated amount of CO_2 (mg CO_2 /kg).

As the initial AA content in each product is usually not the same, the contents were normalized against the initial values and expressed as follows:

$$AA_{rel} = 100 \left(\frac{AA_t}{AA_0} \right) \quad (2)$$

where AA_{rel} is the relative ascorbic acid content (%).

Substituting Eq. (2) into Eq. (1) gives, we get

$$AA_{rel} = 100 \exp(-\beta AR_{CO_2}) \quad (3)$$

5. Statistical analysis

The respiration rate and the AA content were determined in three replications with different samples. The value of parameter β in Eq. (3) of each selected vegetable was estimated by the least squares method.

An F -test for homogeneity of regression coefficients was used to analyze the differences in each β value among the selected vegetables at the 5% level according to the method of Gomez and Gomez (1984).

III Results and discussion

1. Respiration rate and ascorbic acid changes

The respiration rates and AA content changes in cabbage, cauliflower, spinach, and green pepper during storage at different temperatures are presented in Fig. 1 to 4, respectively. The CO_2 production rates differed greatly among the tested vegetables; at the same storage temperature, they decreased in the following order (highest to lowest): cut cauliflower, spinach leaves, cut green cabbage, and cut green pepper. Similar differences have also been reported in previous studies (Tewfik and Scott, 1954; Jacxsens et al., 2000). In addition, the CO_2 production rate in all samples was suppressed more at lower than at higher temperatures. This indicates tissue metabolism is accelerated at higher temperatures. The respiration rate of all the vegetables, except green pepper, decreased until about 2 days after storage at all storage temperatures, and it was then maintained for the remaining storage period (Fig. 1a to 3a). On the other hand, there was a gradual increase in the respiration rate of cut green pepper stored at 5°C was observed (Fig. 4a). Green pepper is

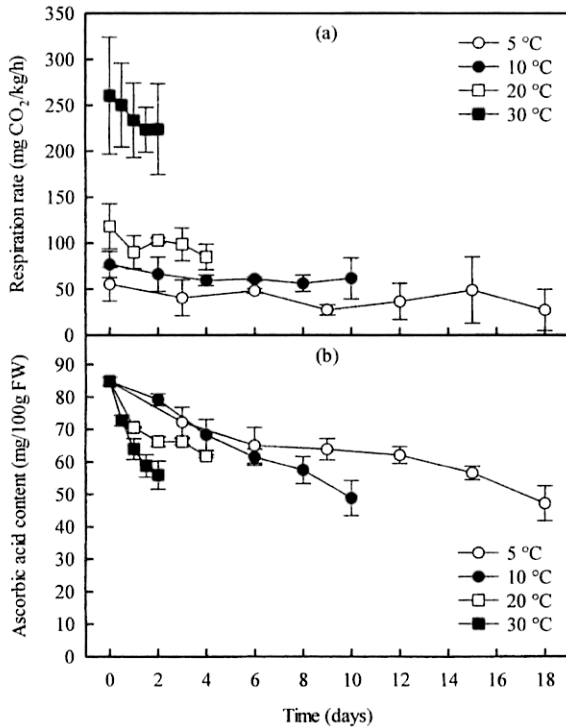


Fig. 3 Changes in respiration rate (a) and ascorbic acid content (b) of stored spinach leaves at various temperature conditions. Each vertical bar represents the standard deviation (n=3).

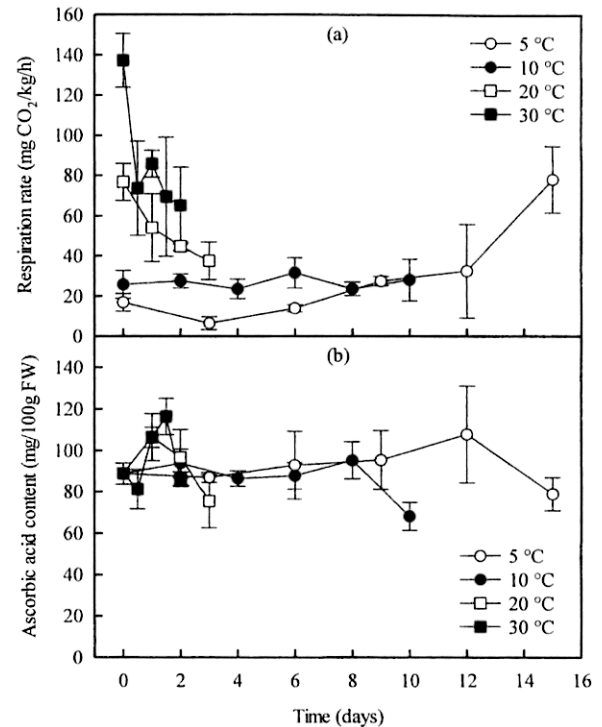


Fig. 4 Changes in respiration rate (a) and ascorbic acid content (b) of stored cut green pepper at various temperature conditions. Each vertical bar represents the standard deviation (n=3).

classified as a chilling sensitive commodity. At temperatures lower than the chilling temperature, these products demonstrate characteristic respiration in response to the chilling stress. An increase in the respiration rate of cut green pepper stored at 5°C has also been reported by Kang and Lee (1997). In this experiment, the visual injury symptoms were observed on 15 days after storage.

Changes in the AA content in cabbage, cauliflower, spinach and green pepper during storage at various temperatures are shown in Fig. 1b to 4b. We observed a wide difference in the content and stability of AA among the vegetables was also observed. The AA content decreased with an increase in the storage period for cabbage, cauliflower and spinach (Fig. 1b to 3b). The reduction in the AA content was slow at low storage temperatures but rapid at high storage temperatures, because the chemical reaction rate is dependent on temperature, and AA degradation is a biochemical reaction. Therefore, temperature management is considered an important tool for delaying deterioration of the quality of fresh produce, particularly with regard to AA content (Wills et al., 1984; Kailasapathy and Koneshan, 1986; Lee and Kader, 2000; Tulio Jr. et al., 2002). However, the AA content in cut green pepper stored at 5 and 10°C did not present obvious changes before decreasing on 15 and

10 days after storage, respectively. But for the cut green pepper stored at 20 and 30°C, the AA content increased during the first day and then decreased throughout the remaining storage period (Fig. 4b). In general, although the AA content in fresh produce decreases with time after harvest (Wills et al., 1984; Lee and Kader, 2000), studies have also shown constant or increasing levels of AA in potatoes (Mondy and Leja, 1986), fresh-cut potatoes (Tudela et al., 2002), green peppers (Jimenez et al., 2003; Gonzalez-Aguilar et al., 2004), strawberries (Cordenunsi et al., 2005) and fresh-cut celeries (Vina and Chaves, 2006). Moreover, Reyes et al. (2007) showed that the change in reduced AA content in fresh produce after wounding was different among commodities and suggested that the retention of AA might depend on the type of tissue. Therefore, our observation in the AA change of cut green pepper is plausible.

2. Relationship between AA_{rel} and AR_{CO_2} of selected vegetables

To better understand the AA change in samples with increasing AR_{CO_2} , the measured AA content was normalized against the initial value for each sample. Then, AA_{rel} of each product was plotted against the accumulated amount of CO_2 production (AR_{CO_2}), as shown in Fig. 5 to 8. The AA_{rel} changes in cabbage, cauliflower and spinach are presented in Fig. 5 to 7,

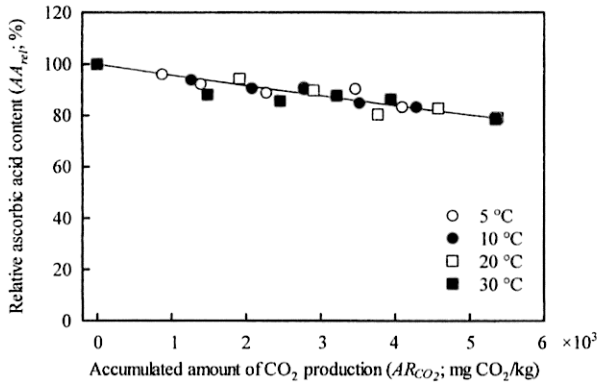


Fig. 5 The relationship between the relative ascorbic acid (AA_{rel}) content and the accumulated amount of CO_2 production of cabbage stored at different temperature conditions. The curve in this figure represents the values calculated by Eq. (3) with the β value in Table 1.

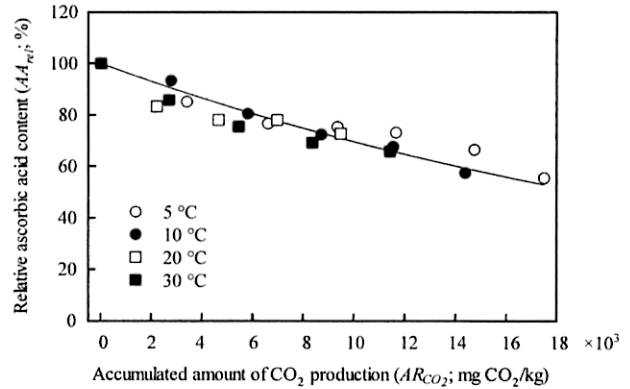


Fig. 7 The relationship between the relative ascorbic acid (AA_{rel}) content and the accumulated amount of CO_2 production of spinach leaves stored at different temperature conditions. The curve in this figure represents the values calculated by Eq. (3) with the β value in Table 1.

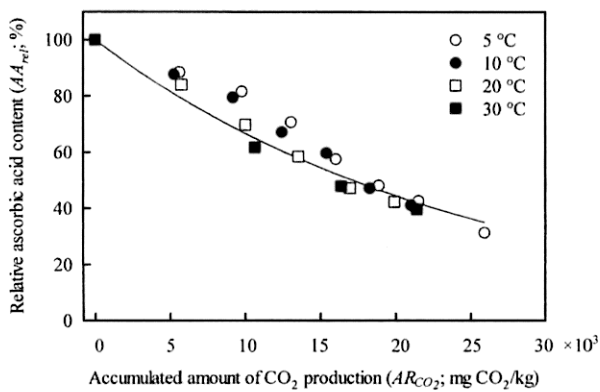


Fig. 6 The relationship between the relative ascorbic acid (AA_{rel}) content and the accumulated amount of CO_2 production of cauliflower stored at different temperature conditions. The curve in this figure represents the values calculated by Eq. (3) with the β value in Table 1.

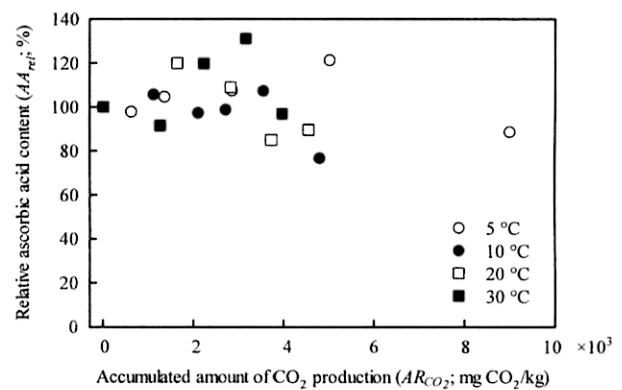


Fig. 8 The relationship between the relative ascorbic acid (AA_{rel}) content and the accumulated amount of CO_2 production of green pepper stored at different temperature conditions.

respectively. It is clearly shown that AA_{rel} decreases with increasing AR_{CO_2} in all samples. In contrast, the relationship between AA_{rel} and AR_{CO_2} for green pepper was different from the above-tested commodities and was scattered as shown in Fig. 8. The accumulated amount of CO_2 production noted in this study can be assumed to be related to the degradation of AA in the samples of cabbage, cauliflower and spinach, but not green pepper.

Respiration is a process that produces free radicals, and living plants have an antioxidant system that prevents cell damage caused by such substances. AA scavenges active oxygen species and is a major antioxidant in cabbage, cauliflower (Kurilich et al., 1999; Podsedek, 2007) and spinach (Gil et al., 1999). In our

study, green cabbage, cauliflower and spinach showed a close relationship between AA_{rel} and AR_{CO_2} . This implies that the respiration process has an indirect effect on the degradation of AA. One possible way that AA was decomposed is by acting as the scavenger of free radicals produced in respiration. However, in fresh produce as well as organic compounds such as other vitamins, pigments and phenolics play a role in radical scavenging. Green pepper is characterized by being very rich in phenolics (Navarro et al., 2006; Conforti et al., 2007; Sun et al., 2007), which can retard or inhibit lipid peroxidation by acting as radical scavengers. Considering the fact that the time dependence of AA degradation was not clearly observed in green pepper, phenolics might be used for scavenging radicals in place of AA. Therefore, the relationship between AA change and respiration could not be deter-

Table 1 The estimated parameter β in Eq. (3) for each stored product

Genus	Products	$\beta \pm \text{SE}$ ($\times 10^{-5}$; kg/mg CO ₂)	R ²	p-value
<i>Brassica</i>	Broccoli	4.44 \pm 0.16 ^a	0.86	< 0.001
	Cabbage	4.39 \pm 0.17 ^a	0.88	< 0.001
	Cauliflower	3.98 \pm 0.12 ^a	0.94	< 0.001
<i>Spinacia</i>	Spinach	3.46 \pm 0.14 ^b	0.89	< 0.001
<i>Capsicum</i>	Green pepper	0.44 \pm 0.71	0.01	0.395

In each β parameter, values followed by the same letter are not significantly different at the 5% level of significance. Data for broccoli was obtained from Techavuthiporn et al. (2008).

mined for green pepper.

The β values of Eq. (3) for each selected vegetable were estimated by the least squares method and are listed in Table 1. The possibility of obtaining a result (*p*-value) of broccoli (data from Techavuthiporn et al., 2008), cabbage, cauliflower and spinach indicates that our simple equation appropriately expresses the relationship between AA_{rel} and AR_{CO_2} . Thus, Eq. (3) is also applicable in cabbage, cauliflower and spinach in addition to broccoli, as discussed previously (Techavuthiporn et al., 2008). The proposed equation would be a useful in designing the optimum distribution and storage conditions for these products. Since the equation expresses on the relative AA content change as a function of respiration, we can calculate the percent residual AA against the time of harvest as well every point after harvest without knowing the absolute initial AA content and only knowing the amount of CO₂ production for the target commodities by means of published respiration data and models. In cut green pepper, however, a high *p*-value indicated that this equation could not sufficiently describe the changing AA_{rel} .

Moreover, the difference in β values among the selected vegetables was evaluated by the *F*-test for homogeneity of regression coefficients. It was found that there was no significant difference in β values among broccoli, cabbage and cauliflower, which belong to the *Brassica* genus. The β value for spinach (*Spinacia*) was significantly different from those of other commodities (*p* < 0.05). These results suggest that the β parameter would depend on the kind of genus rather than the commodity itself. This implies that the prediction of AA content in *Brassica* products, such as broccoli, cabbage and cauliflower, might be possible by substituting their own respiration data into Eq. (3) with a common β value (4.27×10^{-5} ; kg/mg CO₂). Our study only considered variation in the genus *Brassica*, and further research on a wide range of genera is required to confirm that the β value depends on the genus of product. Moreover, a limitation of our model was observed in the case of green pepper. Since the AA change in green pepper was not related to its respiration, the group of products for which the AA

content could not be predicted by our model should be investigated further.

IV Conclusion

In this study, to confirm the applicability of our empirical AA model as a function of respiration to a wide range of vegetables, the relationship between the AA content of broccoli, cabbage, cauliflower, spinach and green pepper and their respiration characteristics was investigated. The change in AA content of broccoli, cabbage, cauliflower and spinach was related to respiration, and was appropriately expressed by our AA model. The β parameter values of broccoli, cabbage and cauliflower did not differ significantly, but that of spinach was different. This research indicated that our respiration-based AA prediction model could be applied not only broccoli (previous study) but also cabbage, cauliflower and spinach. The AA content in the above-tested species under conditions of fluctuating temperature and gas composition, which often occurs in a distribution chain, would be able to predict by substituting their respiration data obtained from previous models into our empirical equation with an estimated β parameter. However, the change in AA content for green pepper was not related to its respiration, which was the one limitation of our model. In future research, while β values must be continually obtained for various product genera, classification of the products to which our model can and cannot be applied is also required.

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「研究論文」

数種青果物に対する呼吸に基づくアスコルビン酸含量変化予測モデルの適用性

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要 旨

先に提案した呼吸量を関数としたブロッコリーの総アスコルビン酸 (AA, 還元型および酸化型アスコルビン酸の総量) 含量変化予測モデルの広範な青果物における適用性を検証するため, キャベツ, カリフラワー, ホウレンソウおよびピーマンを対象に, 種々の温度環境 (5, 10,

20, 30°C) に貯蔵した際の, AA 含量変化と CO₂ 排出速度との関係について検討した。AA 含量の初期値に対する割合 (AA_{rel} , %) を積算呼吸量 (AR_{CO_2}) に対してプロットした結果, キャベツ, カリフラワーおよびホウレンソウの AA_{rel} は AR_{CO_2} の増加に伴い減少し, これらの関係は先に提案したモデル式 ($AA_{rel} = 100 \exp(-\beta AR_{CO_2})$) によってうまく表現できた。一方, ピーマンでは上記の関係は認められず, モデルの限界性が示された。また, 回帰係数 (パラメータ β) の同等性について統計解析したところ, *Brassica* 種であるブロッコリー, キャベツ, カリフラワーの間では有意差がなかったが, *Spinacia* 種であるホウレンソウの β 値は, 他の品目と有意に異なった。本研究により, 提案したモデルはブロッコリーのみならず他の品目にも適用できることが示され, これら品目の最適な流通設計を行うための有用なツールとなろう。

[キーワード] アスコルビン酸, 呼吸速度, モデリング, *Brassica*, *Spinacia*, *Capsicum*

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