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A New Method for Estimation Quality Deterioration of Agricultural Products

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Abstract
A new method has been development to measure the amount of 1, 3-diethyl-2-thiobarbituric acid (DETBA)-malondialdehyde (MDA) adduct as an index of lipid peroxidation. The DETBA-MDA adduct is extracted with ethyl acetate and the amount is measured by spectrofluorometry of high-performance liquid chromatography with a fluorescence detector. The DETBA method which is highly sensitive, and specific, is as simple as the traditional colorimetric TBA method, and enables to measure DETBA values from several agricultural products. The use of the DETBA method enabled to discriminate slight differences in garden peas with external deterioration that was hardly recognizable with the naked eye. The increase in the DETBA values during storage of the garden peas was closely related to the change in surface color and the decrease in the contents of both chlorophyll and reduced form of (or total) ascorbic acid contents. Similar observation was also made in sweetpotatoes. These results suggest that the DETBA method is suitable for estimating quality deterioration during the storage of agricultural products.

Discipline: Experimental apparatus and method/ Postharvest technology
Additional key words: 1, 3-dienthy1-2thiobarbituric acid, lipid peroxidation, storage, garden pea, sweetpotato

Introduction

Monitoring and control of oxidative deterioration during the storage and processing of agricultural products have become increasingly important due to the growing demand for foods of high quality. In connection with the quality deterioration, lipid peroxidation has attracted much attention, since lipid peroxidation in foods leads to the developement of off-flavors, bitter taste, and toxic substances, as well as nutritional damage[4,5,8]. The levels of lipid peroxidation have often been measured by the 2-thiobarbituric acid (TBA) method as an index of storage stability and quality of fats, oils, and other lipid-rich foods[9]. However, changes in agricultural products with low lipid contents are poorly documented because of the lack of suitable detection methods.

Recently, we have improved the common TBA method and developed a highly sensitive, specific, and simple method for measuring lipid peroxidation, namely the 1,3-diethyl-2-thiobarbituric acid (DETBA) method[10]. This method which could be applied to various agricultural products with very low TBA values, such as vegetables, fruits and potatoes[10], enabled to examine readily the changes in the lipid peroxidation levels during storage[3].

Thus, this paper introduces the newly developed method for estimating the quality deterioration of agricultural products.

Procedure of DETBA method

The basic procedure of the DETBA method has been described previously[10]. To minimize autooxidation and/or enzymatic oxidation by lipoxygenase etc., during sample preparation, the modified method currently used in our laboratory is as follows (Fig. 1).

Plant tissues were homogenized with 20 mM butylated hydroxytoluene (BHT) in ethanol using a Polytron homogenizer. An aliquot (less than 0.2 ml) of the homogenate (equivalent to less than 40 mg of plant material) was transferred to a screw-capped tube containing 0.4 ml of 4% sodium dodecyl sulfate (SDS) and 0.2 ml of distilled water, and the mixture was finally made up to 0.8 ml with 20 mM

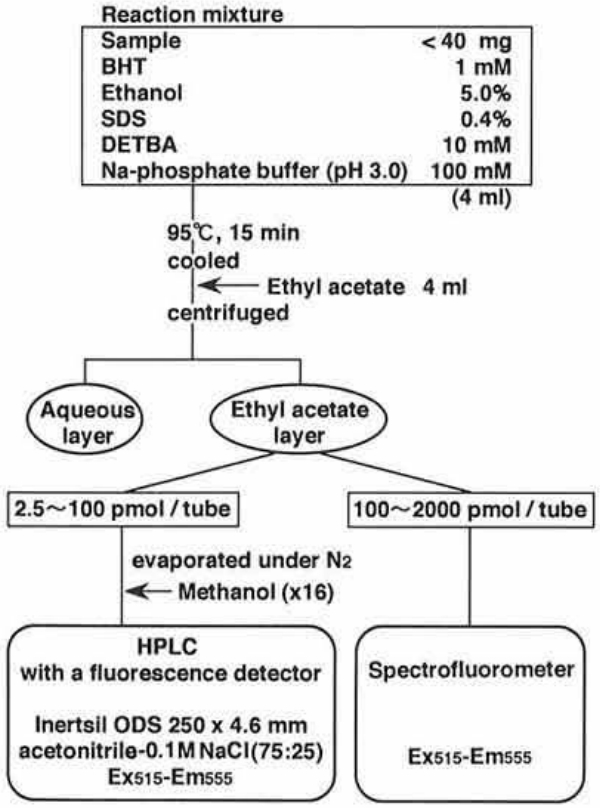

Fig. 1. Flow diagram for the measurement of DETBA-MDA adduct

BHT in ethanol. After the addition of 3.2 ml of 12.5 mM DETBA in a sodium phosphate buffer (0.125 M, pH 3.0) warmed to 50°C, the solution was mixed and heated in a water bath at 95°C for 15 min, and then cooled quickly with running tap water. To extract the DETBA-malondialdehyde (MDA) adduct, 4 ml of ethyl acetate was added, and the mixture was shaken vigorously. After centrifugation at 2,000 rpm for 10 min at 20°C, the fluorescence intensity of the organic layer was measured at an excitation wavelength of 515 nm and emission wavelength of 555 nm ($Ex_{515}-Em_{555}$).

The DETBA value was calculated as nanomoles of MDA per gram of fresh weight from a calibration curve prepared with 1,1,3,3-tetraethoxypropane as the standard (Fig. 2). A stock standard solution was prepared at the concentration of 10 nmol/ml of 1,1,3,3-tetraethoxypropane in 0.1% methanol and 0.02% sodium azide, and x ml of the stock solution (less than 0.2 ml) was added to a test tube containing 0.2 ml of 20 mM BHT in ethanol, 0.4 ml of 4% SDS and $(0.2-x)$ ml of distilled water. The following procedures were carried out in the same way as for the plant tissue samples.

To prepare samples for the high-performance

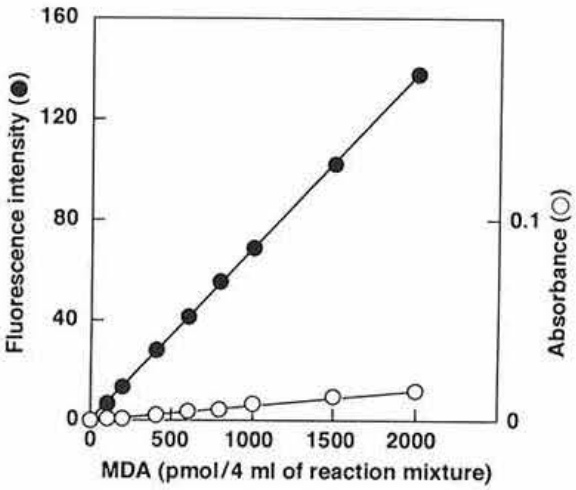

Fig. 2. Calibration curves for the MDA standard obtained by spectrofluorometry and spectrophotometry
(●) Fluorometry of the DETBA-MDA adduct ($Ex_{515}-Em_{555}$),
(○) Spectrophotometry of the TBA-MDA adduct (A_{532}).

liquid chromatography (HPLC) analysis, an ethyl acetate extract (2.4 ml) containing the DETBA-MDA adduct was transferred to another tube and evaporated under nitrogen. The residue was dissolved in 150 μl of methanol, and 10 μl of the sample was applied to HPLC under the following conditions: column, Inertsil ODS (5 μm particle size, 250 × 4.6 mm i.d.); mobile phase, acetonitrile-0.1 M sodium chloride (75 : 25, v/v); flow rate, 1.0 ml/min; detection, $Ex_{515}-Em_{555}$. To obtain the calibration curve, DETBA-MDA adduct prepared from 0.1-20 pmol of MDA in 10 μl was injected in the HPLC system.

Advantages of DETBA method

The DETBA method has several advantages compared to the traditional colorimetric TBA method. The first advantage is the high specificity. It has been reported that when the TBA method was applied to certain foods, a yellow color was produced instead of the typical pink color[1,9]. If the color is sufficiently intense, it may overlap with the TBA-MDA absorption and give an erroneously high value. The substances responsible for the appearance of the yellow color include monosaccharides and disaccharides[2,7,9], which are common components in agricultural products. In our method, the DETBA-MDA adduct was extracted with ethyl acetate and the amount was measured by spectrofluorometry.

Ethyl acetate can eliminate many yellowish coexisting substances. As a result, the substances interfering with the TBA method, such as glucose, fructose, sorbitol, mannitol, sucrose, lactose, maltose, starch, glycerin, glycine, methionine and arginine did not cause any disturbance up to 16 mg/4 ml of reaction mixture (Table 1). Although galactose, mannose, pectin, glutamic acid, and bovine serum albumin may interfere (Table 1), the interference can be easily prevented by limiting the size of the samples added to the reaction mixture. The recommended sample size is less than 40 mg of plant material/4 ml of reaction mixture.

The use of fluorometry as a detection technique is also important to measure accurately the amount of DETBA-MDA adduct from plant materials. Fig. 3 shows HPLC chromatograms of DETBA-reactive substances from apple, strawberry, spinach and pea. The peak value of the DETBA-MDA adduct appeared at a retention time of 9 min. The other small peaks eluted before this value were observed at 539 nm. Those peaks were also detected at 450 nm, indicating the presence of yellowish substances. The percentage of the DETBA-MDA adduct in all the DETBA-reactive substances detected

Table 1. Substances interfering with measurement of the DETBA value by spectrofluorometry

Non-interfering substances	Interfering substances
Glucose	Galactose (8-16 mg/4 ml)
Fructose	Mannose (8-16 mg/4 ml)
Sorbitol	
Mannitol	
Sucrose	
Lactose	
Maltose	
Starch	Pectin (4-16 mg/4 ml)
Glycerin	
Glycine	
Methionine	Glutamic acid (16 mg/4 ml)
Arginine	Albumin (16 mg/4 ml)

Values in parenthesis indicate the substance concentrations showing a fluorescence intensity equivalent to MDA of >100 pmol/4 ml of reaction mixture.

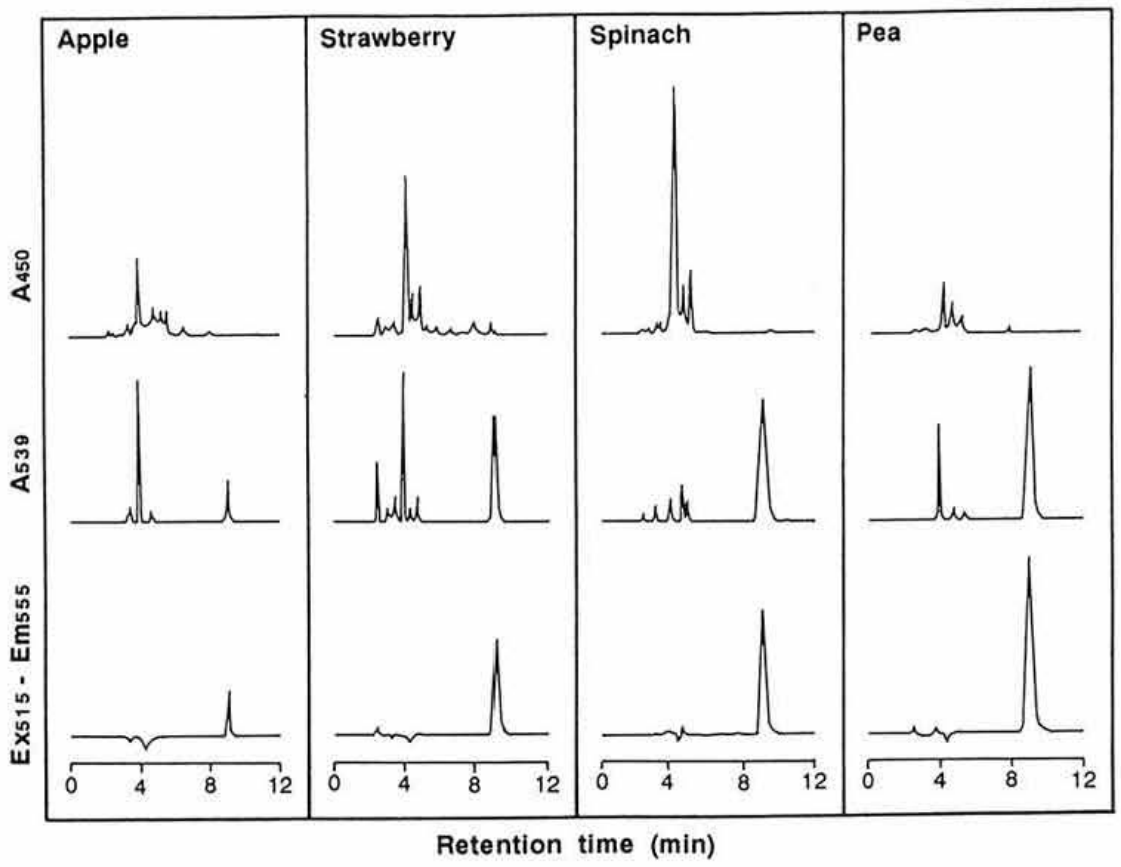

Fig. 3. HPLC chromatograms for the reaction product of agricultural products with DETBA The DETBA-MDA adduct was prepared from apple (40 mg), strawberry (40 mg), spinach (40 mg), or pea (0.4 mg) in the reaction mixture in a total volume of 4 ml. Each sample was concentrated 16-fold, and 10 μl was subjected to HPLC.

at 539 nm was only 36% for apple, 45% for strawberry, and 75% for spinach and pea. On the contrary, the percentages exceeded 98% at $Ex_{515}-Em_{555}$. These results indicate that the fluorometric detection method is superior to the colorimetric detection method in its specificity for the detection of the DETBA-MDA adduct.

Another advantage is the high sensitivity. The fluorometric DETBA method enabled to detect the MDA which could be hardly detected when the colorimetric TBA method was applied (Fig. 2). The use of HPLC with a fluorescence detector is also a more sensitive method for measuring the amount of DETBA-MDA adduct. According to the recommended sample size (<40 mg of plant material/4 ml of reaction mixture), the detectable amount of the DETBA-MDA adduct from plant materials was more than 2.5 nmol/g fresh weight (=100 pmol/40 mg of plant material/4 ml of reaction mixture) and more than 0.0625 nmol/g fresh weight (=2.5 pmol/40 mg of plant material/4 ml of reaction mixture) by spectrofluorometry and HPLC with a fluorescence detector, respectively. Thus, an amount of DETBA-MDA adduct ranging from 0.8-18.0 nmol/g fresh weight can be easily detected from vegetables, fruits and potatoes by using both or either techniques (Table 2). In addition, there was a positive correlation between the values obtained by spectrofluorometry and those by HPLC with a fluorescence detector.

Table 2. DETBA values from agricultural products measured by spectrofluorometry and HPLC with a fluorescence detector

Agricultural product		DETBA value (nmol/g of fresh weight)	
		Spectrofluorometry	HPLC with a fluorescence detector
Pulses	Soybean	611.12	609.93
	Pea	136.96	134.91
	Garden pea	11.22	10.87
Vegetables	Spinach	11.28	11.16
	Green pepper	4.19	4.16
	Carrot	3.21	3.15
	Tomato	2.94	2.92
	Onion	<2.50	1.91
Fruits	Strawberry	3.58	3.52
	Lemon	3.21	3.02
	Banana	<2.50	1.49
	Apple	<2.50	0.92
Potatoes	Potato	18.01	17.77
	Sweetpotato	<2.50	1.47
	Satoimo	<2.50	0.77

Application of DETBA method for the estimation of quality deterioration of agricultural products

1) Garden peas

We selected garden peas (*P. sativum* subsp.

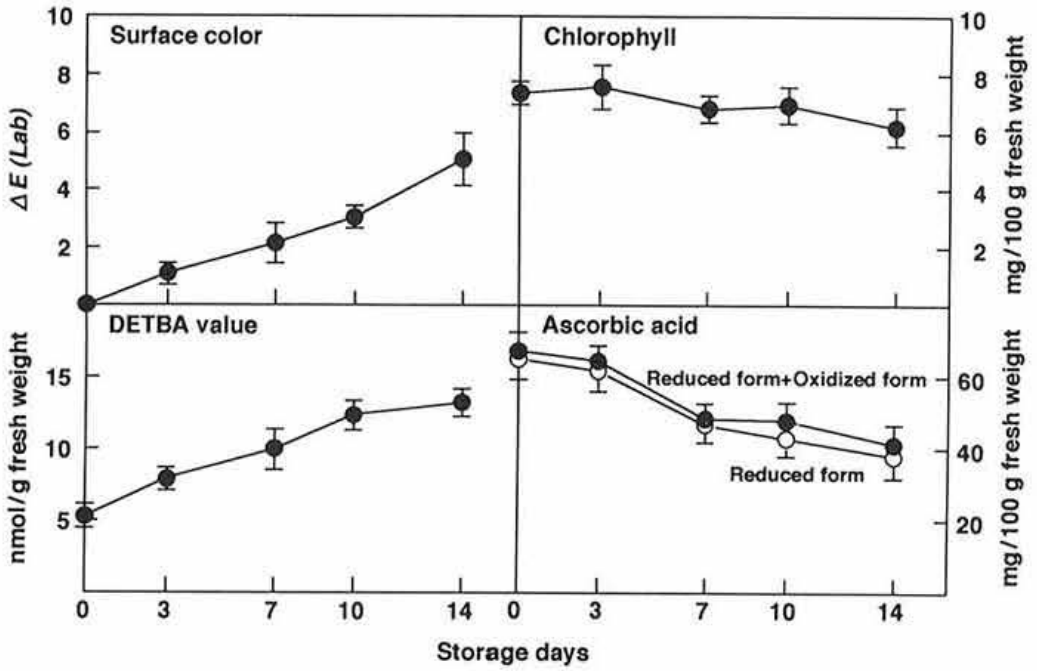

Fig. 4. Relationship between DETBA-MDA adduct and surface color, chlorophyll, and ascorbic acid contents of stored garden peas

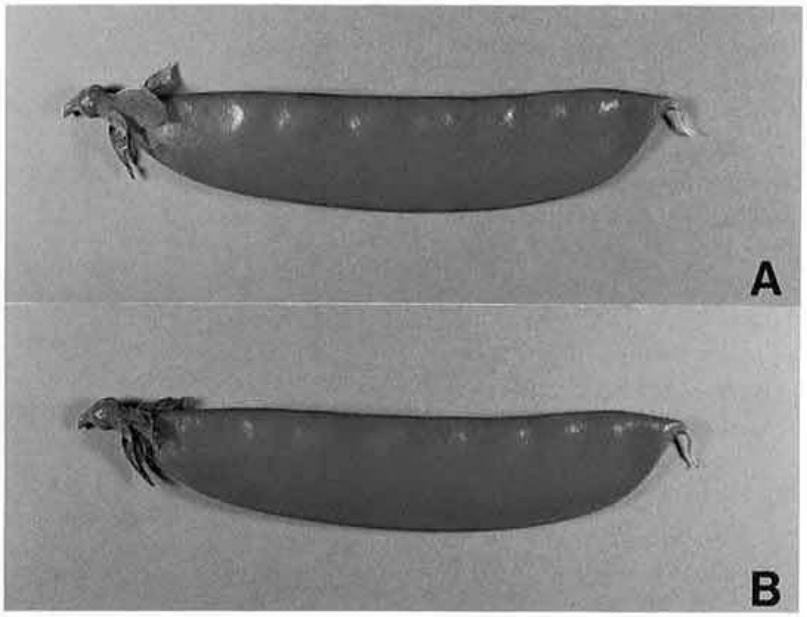

Plate 1. Deterioration of external quality of stored garden peas
A; Non-stored, B; Sored for 10 days in the dark at 5°C.

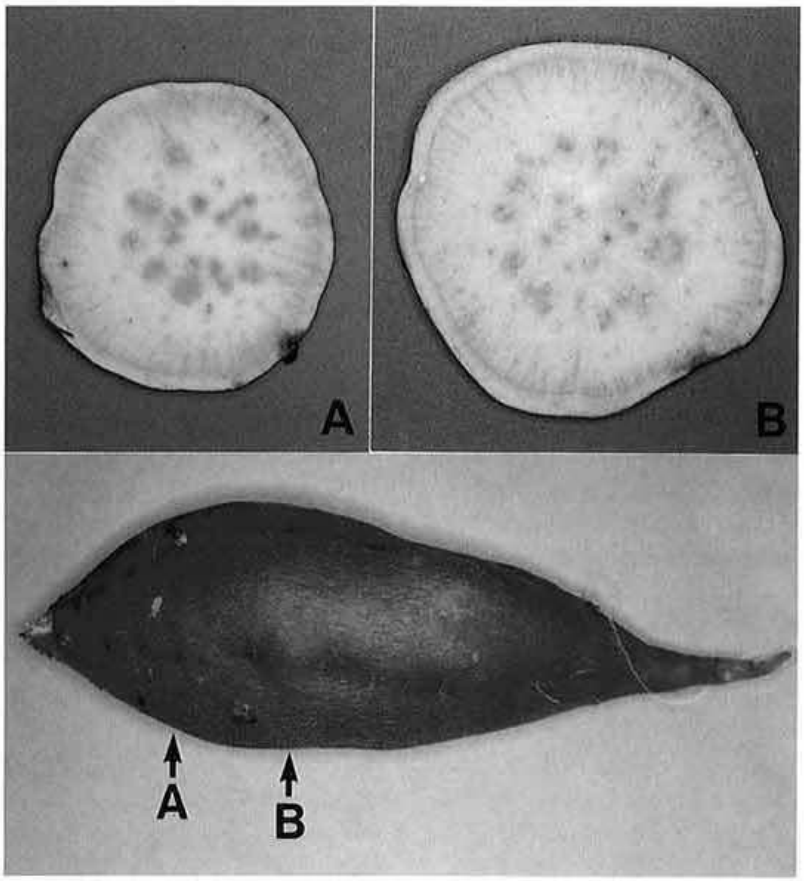

Plate 2. Cut surfaces of sweetpotato cultivar Kokei 14 stored for 8 months at 15-20°C in the dark

hortense Asch.) as an agricultural product with a low lipid content and determined whether quality deterioration during storage of garden peas could be estimated by the developed DETBA method. When 30 pods of garden peas were divided into 5 groups of 6 pods and stored at 5°C in the dark for 0, 3, 7, 10, and 14 days, respectively, the DETBA value increased during storage (Fig. 4). Plate 1 illustrates 2 pods of garden peas with different DETBA values. The values were 5 nmol/g fresh weight (Plate 1-A) and 13 nmol/g fresh weight (Plate 1-B). Thus the DETBA method enabled to discriminate slight differences in garden peas with external deterioration that would have hardly been recognizable with the naked eye.

Then we examined the relationship between the DETBA value during storage and other deterioration indexes, such as surface color, ascorbic acid, and chlorophyll content. Here, surface color values before (L_0, a_0, b_0) and after (L, a, b) storage of the garden peas were directly measured using a color difference meter, and changes in surface color (Hunter color difference, $\Delta E(Lab)$) during storage were obtained from the following equation:

$$\Delta E(Lab) = \{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2\}^{0.5}$$

Reduced form of and total ascorbic acid contents were measured by HPLC analysis, and total chlorophyll contents were calculated by measuring the absorbance at 645 nm and 663 nm according to Arnon's method.

As shown in Fig. 4, the increase in the DETBA values was closely related to the changes in surface color and the decreases in both chlorophyll and reduced form of (or total) ascorbic acid contents. Based on the positive correlations between the DETBA values and other deterioration indexes, it is suggested that the DETBA method is suitable for estimating the quality deterioration of stored garden peas.

2) Sweetpotatoes

We determined furthermore whether the DETBA method was suitable for estimating the quality deterioration of sweetpotatoes. In this case, the DETBA values were measured by HPLC with a fluorescence detector, due to the very low DETBA values (Table 2). Sweetpotatoes tested were Kokei 14 stored for 8 months at 15-20°C in the dark. Plate 2 shows a root and the cut surfaces. Quality deterioration was more evident in surface A than in surface B. The DETBA values were 1.64 nmol/g fresh weight for surface A and 0.64 nmol/g fresh weight for surface B. In addition, the increase in the DETBA values was related to the decrease of the contents of the reduced form of (or total) ascorbic acid (Fig. 5). Thus, the DETBA method enabled to estimate the quality deterioration of sweetpotatoes as well as garden peas.

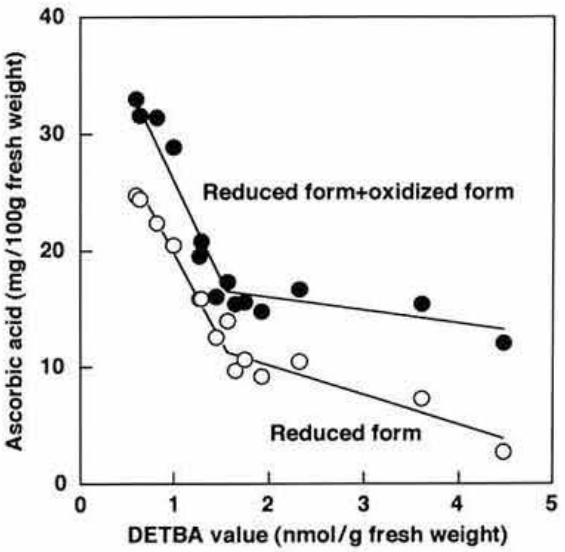

Fig. 5. Relationship between DETBA-MDA adduct and ascorbic acid contents of sweetpotato cultivar Kokei 14 stored for 8 months at 15-20°C in the dark

3) Other agricultural products

In this study, we clearly demonstrated that lipid peroxidation was induced even in garden peas and sweetpotatoes with low levels of lipids. We also obtained positive correlations between the lipid peroxidation index and other deterioration indexes, indicating that the DETBA method was suitable for estimating the deterioration of stored garden peas and sweetpotatoes. Since the use of this technique enables to evaluate quality deterioration, further studies are in progress to determine to what extent the DETBA value increases along with quality deterioration during the storage and processing of other agricultural products (e.g. soybean[6] and rice). For the application of the DETBA method to other agricultural products, one should pay attention to the following points: 1) To verify whether no overestimation of DETBA-MDA occurs at Ex_{515}-Em_{555}. The influence of coexisting substances affecting the DETBA value can be evaluated by HPLC in the same way as shown in Fig. 3. 2) To determine whether the DETBA-MDA adduct can be detected

by either a spectrofluorometer or HPLC with a fluorescence detector. In the case of samples with a DETBA value of >2.5 nmol/g fresh weight, the measurement of the DETBA value is rapid with spectrofluorometry, because spectrofluorometry is superior to HPLC with fluorescence detector due to its simplicity. 3) The size of the sample added to the reaction mixture plays a major role in the successful application of the method and our studies showed that an amount of less than 40 mg of fresh weight was adequate.

The DETBA method we developed is highly sensitive and specific. It is as simple as the traditional colorimetric TBA method, and it enables to measure DETBA values from several agricultural products. However, the DETBA value is not an absolute value but only a relative value of hydroperoxide contents as in the case of the TBA value. If this aspect is recognized, the DETBA value can be considered to be a convenient and sensitive index of quality deterioration of various agricultural products.

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