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Influence of Gas Mixture Fumigation of Ethyl Formate and Carbon Dioxide on Several Characteristics of Fresh Fruits and Vegetables (Bananas, Pineapples, Strawberries, Grapefruits, Satsuma mandarins, Squashes, and Other Vegetables)

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Abstract: Several characteristics of bananas, pineapples, strawberries, grapefruits, Satsuma mandarins, squashes, string beans, parsley, and broccoli were investigated with the aim of determining whether gas mixture fumigation with ethyl formate (EF, C₂H₅O)₂, 75.2 mg/l, and carbon dioxide (CO₂), 374.9 mg/l, for 3 h at 15°C causes phytotoxicity. EF sorption into a commodity was concluded to depend on the commodity's surface area. Fumigated bananas, pineapples, strawberries, and squashes showed no difference in weight, hardness, color, soluble solid content, or flavor compared with untreated controls except for the banana surface color and squash flavor. Some color change was observed on the surface of bananas because of insufficient evaporation of liquefied EF; fumigated squashes were considered preferable in a flavor test. EF and CO₂ gas mixture did not appreciably interfere with banana ripening. Characteristics including color (L*a*b* color space) of fumigated grapefruits and Satsuma mandarins were not different from those of untreated fruits, but Satsuma mandarins were softer 7 days after fumigation. EF gas concentrations for parsley and broccoli were substantially lower than that for string beans from beginning just after treatment. Parsley and broccoli mostly lost weight after 7 days of storage. The color of fumigated parsley changed markedly, accompanied by severe wilting, while broccoli showed no change. No negative effects were observed on the flavor of fumigated string beans and broccoli, whereas fumigated parsley showed its smell turned stronger and its leaves were coated with a viscous liquid.

Key words: ethyl formate, carbon dioxide, phytotoxicity, fruit quality, quarantine treatment

Introduction

Methyl bromide (MB) is one of the important fumigant for plant quarantine, but the Montreal Protocol urges the reduction of MB use and emissions into the atmosphere even for quarantine and pre-shipment (QPS) purposes (UNEP, 2009). According to statistical reports on plant quarantine in Japan, the major uses of MB for QPS purposes in Japan in 2007 were for logs, grains, and fruits and vegetables. In 2010, however, amounts used for fumigation of logs, grains, beans, and vegetables decreased by 52%, 71%, 56%, and 68%, respectively, while those for fresh fruits increased by 110% over the 2007 values (PPS, 2010). Much research on the development of alternative fumigants to MB has been conducted for logs, including wood packaging materials, and for grains, but not enough research has been conducted for plant quarantine treatment of fresh fruits and vegetables (Misumi et al., 2009). Accordingly, the development of alternative treatments to MB for fresh fruits and vegetables is increasingly urgent.

Fumigation using a mixture of ethyl formate (EF) with carbon dioxide (CO₂) is commercially used under the name of Vapormate™ (EF 16.7% and CO₂ 83.3% (w/w)) in various countries. It is also a naturally existing material that is known as a Generally Recognized as Safe (GRAS) food additive. In several fruits and vegetables such as lettuce, however, some injury has been reported following EF fumigation under certain conditions (Simpson et al., 2007; Stewart and Mon, 1984). We conducted phytotoxicity tests for five types of fresh fruits, squashes, and other vegetables (string beans, parsley, and broccoli) to evaluate the influence of gas mixture fumigation with EF and CO₂ on their quality.

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Materials and Methods

1. Test fruits and vegetables

All test fruits and vegetables were chosen from commodities generally imported into Japan and readily purchased in domestic markets. Green bananas (Musa sp., Cavendish banana) and pineapples (Ananas comosus) imported from the Philippines were purchased from a wholesaler one or two days before fumigation in July and November 2010. Strawberries (Fragaria sp., variety Sachiokika) harvested in Saga prefecture, grapefruits (Citrus X paradisi) imported from South Africa and Satsuma mandarins (Citrus unshiu) harvested in Ehime prefecture were also purchased from a wholesaler the day before fumigation on February, August, and December 2011, respectively. Squashes (Cucurbita maxima, variety Kuriyutaka) harvested in Kagoshima prefecture, was purchased from a wholesaler in June 2011 the day before fumigation. Other vegetables [string beans (Phaseolus vulgaris, from Okinawa prefecture), parsley (Petroselinum crispum, from Ibaraki prefecture) and broccoli (Brassica oleracea var. italica, from Kagawa prefecture)] purchased from a market in 2011 were also used in tests for a preliminary study.

2. Fumigation

All fruits and vegetables were fumigated in acrylic fumigation boxes (W 30 x D 33 x H 45 cm (external dimension), internal volume: 29.5 l, equipped with gas injection and sampling ports, temperature probe, stirrer bar, pressure gauge, and air inlet and exhaust valves) placed in a temperature-controlled fumigation room at 15°C. Dosage rates of both EF and CO₂ were determined assuming the mixture rate (%) in a commercially available gas cylinder (Vapormate™). EF 16.7%; CO₂ 83.3%, w/w. For fumigation conditions in this study, we selected a dosage rate of 75.2 mg/l EF and 374.9 mg/l CO₂ (equivalent to 450 mg/l as a gas mixture), and exposure time and fumigation temperature of 3 h at 15°C, based on the results of tests of scales and mealybugs susceptibility to gas mixtures of EF and CO₂ (Misumi et al., 2013). Application of EF and CO₂ was conducted as follows: EF liquid (purity over 98%, Alfa Aesar) collected from a glass vial by syringe was dropped through an injection port onto a filter paper (ca. 150 mm diameter) in a laboratory dish placed in the fumigation box, and CO₂ gas from a gas cylinder was immediately introduced into the box. Just before application, pressure in the fumigation box was reduced to approximately 20 kPa (ABS) and the pressure was then restored to atmospheric pressure 10 min after application. A magnetic stirrer was continuously activated during exposure time to speed EF evaporation and promote gas circulation. Forced aeration (3 l/min) was applied for 1 h after fumigation.

EF and CO₂ gas concentrations in the space inside the fumigation box during fumigation were periodically measured by gas chromatographs (EF: Shimadzu GC-2014 with FID, CO₂: Shimadzu GC-2014 with TCD). A NDIR gas analyzer (UR-126G Komyo rikagaku kogyo) was also used for measuring CO₂ concentration. A temperature recorder (Chino graphic logger CR 1016-A) was used throughout fumigation to monitor temperatures in fumigation boxes. As control plots, untreated materials were placed in another fumigation box in the same temperature-controlled fumigation room and were made to undergo the same procedures as fumigated ones but without dosing by fumigants.

In case of bananas, artificial ripening was applied after fumigation. After treatment with EF and CO₂ for 3 h and aeration for 1 h, the temperature of the fumigation room was increased to 20°C. Green bananas, left in the fumigation boxes, were exposed to this temperature for several hours. After exposure, the green bananas were treated with ethylene gas at 1000 ppm for 24 h at 20°C in the fumigation boxes and then maintained in the room for 6 days at 20°C without humidity control, following the method of Nakamura et al. (1984) and Yoshioka (1985). Ethylene concentration and temperature were monitored by the gas chromatograph and the recorder mentioned above. The degree of ripeness was confirmed by measurement of fruit hardness and sugar content (Brix %) periodically during the ripening and storage process, as described below.

3. Measurement of characteristics of fresh fruits and vegetables

The weight of fruits and vegetables was measured with an electronic scale (PA-6000, A&D Co. Ltd.), and their hardness, except for that of string beans, parsley, and broccoli, was measured as penetrative force by a circular cone of a fruit hardness tester (Model KM, Fujiwara Scientific Co. Ltd.). Measuring points for hardness were, on bananas, three points on the circumference of the parts around the pedicels, equators, and sepals of five fruits; on strawberries, grapefruits, and Satsuma mandarins, two opposite points on the equatorial area of each of five fruits; and on squashes, four points on the pedicel and sepal circle area and the equatorial area of two fruits. The color of the fruit surface and flesh were compared between fumigated and nonfumigated fruits and vegetables by visual inspection and photographs. The color of the fruit surface of grapefruit and Sa-
pineapples

the lowest value of 32 mg/l at a 60 min after fumigation

tions for the other fruits and vegetables changed in the

time at 30 min after application to

may have been immediately adsorbed to the fumigated

commodities in the initial stage of exposure time

for bananas

considered unimportant for the ripening process. Samples were maintained in the storage room for several days and each characteristic was investigated at 1 and 7 days (citrus fruits), or 4, 5, and 7 days (other fruits and vegetables) after fumigation, using the measurement methods described above. The software JMP 8.0 (SAS Institute Inc. 2008) was used for statistical analysis of the data collected in the study.

Results and Discussion

Changes in gas concentrations of bananas, pineapples, strawberries, grapefruits, Satsuma mandarins, and squashes during fumigation with EF and CO₂ at a loading factor of ca. 0.1 kg/l (ca. 0.05 kg/l for strawberry) are shown in Fig. 1.

Gas concentrations for most commodities increased from the measurement time at 30 min after application to 60 min. Because for laboratory use EF was applied as a liquid, it required time to evaporate depending on the conditions of commodities tested. Considering only concentrations following 60 min after dosing, no fruits or vegetables showed high gas loss from 60 to 180 min after dosing. EF may have been immediately adsorbed to the fumigated commodities in the initial stage of exposure time, with absorption unlikely to have been high by the 60-min time point and thereafter. Although all commodities were fumigated at the same rate of 75.2 mg/l EF, the EF concentration of grapefruit changed higher than the dosage rate, indicating that grapefruits are not EF sorptive. Gas concentrations for the other fruits and vegetables changed in the following descending order: squashes, Satsuma mandarins, pineapples, bananas, and strawberries. Strawberries showed the lowest value of 32 mg/l at a 60 min after fumigation, i.e., at less than half of the dosage applied. Considering that the loading factor of strawberries was low, at 0.05 kg/l, it appeared that strawberries are EF sorptive. For almost all commodities, EF gas concentrations were higher in large-sized and lower in smaller-sized commodities. This result may indicate that the gas sorption of EF to commodities depended on the surface area of the commodities loaded into the fumigation boxes. However, there was an exception to this sorption trend. Pineapple fruit has a scale-like surface appearance and was fumigated with one crown leaf bud remaining at the top of the fruit, giving it a relatively large surface area. In addition, the loading factor of pineapple was larger at approximately 1.2 kg/l than that for the other fruits and vegetables of approximately 1.0 kg/l. Nonetheless, the EF gas concentration for pineapple was higher than expected from the trend, and the gas concentration curve was similar to that of banana.

Weight, hardness, surface color, sugar content (Brix %), and flavor of treated bananas, pineapples, strawberries, and squashes compared with those of untreated control fruits are presented in Table 1.

For bananas, weight, hardness, and flavor of fumigated fruits did not differ from those of untreated controls. Although the sugar content of untreated bananas was 2.6% higher than that of treated bananas, the difference was considered unimportant for the reason described below. However, with respect to banana surface color, phytotoxicity was evident in the form of parts of banana surfaces turning brown in a few fruits treated with EF and CO₂. EF liquid and CO₂ gas were sequentially injected into fumigation boxes. Given that the change of color on banana surfaces
occurred on the side facing the injection port used for liquid EF delivery, it may be that the injected liquid EF did not quickly evaporate, exposing one side of the fruit to a high EF concentration and possibly leading to the color change. The commercial Vapormate™ product consists of EF diluted in liquefied CO2 in a gas cylinder, so that evaporation of EF occurs quickly after application. Thus, the change in color on one side of banana would not occur or would be reduced with the use of cylinderized EF and CO2 in commercial fumigation.

For pineapples and strawberries, none of the characters of treated commodities differed from those of untreated controls, even on pineapple crown leaf buds. Simpson et al. (2004) reported that strawberry calyx showed slight to moderate damage on fumigation with 1.6% EF at 24°C. The EF concentration of 75.2 mg/l used in our study is equivalent to 2.4% at 15°C. Strawberries fumigated in our experiment, however, showed no even slight damage to the calyx, although calyx color appeared slightly darker five days after fumigation. It is likely that calyx damage depends on the strawberry variety or fumigation temperature.

For squash, no difference was observed in weight, hardness, and color; however, a significant (P < 0.05, chi-square test) difference was observed in flavor between treated fruits and the untreated control. We considered that this difference depends on individual squash fruits, given that few of the squash were large and heavy enough to satisfy a loading factor of 0.1 kg/l. Most of the flavor testing panel commented that they preferred fumigated to untreated fruits. Thus, it is reasonable to expect that EF and CO2 fumigation would not damage squash quality.

No color change was observed in the inner flesh of any fumigated sample of the four commodities when they were cut for flavor testing.

Bananas imported to Japan are unripe (green) and are artificially ripened after plant quarantine inspection. The influence of EF and CO2 fumigation on the ripening process must be investigated. Changes of hardness and sugar content (Brix %) during ripening are shown in Fig. 2.

The hardnesses of both treated and untreated banana decreased to 1.5 kg at 6 days after fumigation from 3 kg just after fumigation, and no treatment difference was detected. Sugar content of treated and untreated banana was measured as 6% just after treatment, increasing 4 days afterward to 19.5% in untreated and 16.9% in treated fruits, and to 22.3% and 20.1%, respectively, at 6 days after treatment. Considering only hardness and sugar content, it appears that banana fumigated with EF and CO2 ripened more slowly than untreated controls. However, these hardness and sugar content differences were not detected in a triangle test for
flavor by a panel of 19 evaluators, suggesting that EF and CO₂ fumigation would have negligible influence on the banana ripening process.

Citrus fruits are considered to be the primary fruits in the fresh fruit export industry in many countries. MB fumigation is applied in current quarantine treatment for citrus, but this causes browning and other damage to fruits (DeLima, 2011). Alternative chemicals are required for the treatment of citrus fruits; their shelf life is longer than that of other fruits. We carried out a more precise investigation of the characteristics of grapefruits and Satsuma mandarins. Mean weight per fruit, hardness, sugar content (Brix %), and flavor of fumigated and untreated fruits at 1 and 7 days after treatment are presented in Table 2.

The fruit weight of untreated control grapefruit 7 days after treatment was 0.40 kg, slightly lighter than treated fruits, but the difference was likely an artifact of unevenness of fruit size and nonsignificant because of the large variance of the mean. For grapefruit hardness measured 1 day after treatment, a significant difference (Wilcoxon test, \( P < 0.05 \)) was found between treated untreated fruits. Control fruits showed a low hardness of 1.76 kg, whereas treated fruits showed 2.04-2.08 kg hardness; moreover, the equality of variance for hardness was rejected in the statistical test. These results suggest that grapefruit size and condition such as peel thickness were variable and that the selection of control fruits was biased. In contrast, Satsuma mandarin showed softer fruit in fumigated than in controls, with a significant (Student’s t-test, \( P < 0.05 \)) difference in hardness 7 days after treatment. Because the unevenness of fruit size had not been taken into account, gas mixture fumigation with EF and CO₂ was concluded to cause softening of Satsuma mandarins.

No difference was detected for other fruit characteristics with EF and CO₂ fumigation compared to untreated fruit at either 1 or 7 days after treatment.

Surface colors of grapefruits and Satsuma mandarins measured in L*\( \times a'b' \) color space are presented in Table 3.

No change of color was detected in L*\( \times a'b' \) values between treated and untreated fruits for any commodity. Days after fumigation, fumigated fruit turned yellowish (+\( b' \)) and untreated fruit showed increased reddishness (+\( a' \)) in grapefruits. For Satsuma mandarins, reddishness of fumigated fruits was weaker than that of the untreated controls, though only slightly. For grapefruits, color differences (\( \Delta E^*ab \)) between treated and untreated fruits were 3.84 1 day after and 4.44 7 days after treatment, and for Satsuma mandarin were 4.17 1 day and 5.55 7 days after treatment.
treatment. Color differences between 1 and 7 days were not significant for any citrus species (Student's t-test, P > 0.05). EF and CO₂ fumigation would thus not affect the surface color of citrus such as grapefruits and Satsuma mandarins. In addition, the inner flesh of both citrus fruits showed no color change following fumigation with EF and CO₂ when they were cut for the flavor test. As for other kinds of citrus fruits, oranges fumigated with mixtures of EF and CO₂ has been well investigated. The quality of navel oranges after exposure to 1% Vapormate™ for 1 h and cold storage of 5 weeks at 5°C was investigated and no effect on weight loss, peel damage, firmness, titratable acidity, soluble solids content, or decay was found (Mitcham et al., 2011). Navel oranges fumigated with Vapormate™ 420 g/m³ for 4 h at 5 and 17°C and stored 15 days at 6°C were not significantly different from untreated fruit with respect to hardness, color, or soluble solids, although slight surface pitting was observed with fumigation at higher temperatures (Sung et al., 2008). From the results of our study, as mentioned above, Satsuma mandarin softened after 7 days storage following EF and CO₂ fumigation. Citrus fruits that, like Satsuma mandarins, could be peeled easily and may be damaged by EF and CO₂ fumigation at a dosage rate above 420 g/m³ and exposure time longer than 3 hours, although more precise studies are necessary. However, in a comprehensive view, no moderate to significant injury or change in fruit quality was observed in our study for either citrus fruits despite a high dosage rate of 450 g/m³ and 3 h of exposure.

Changes of EF gas concentration during exposure for three kinds of vegetables fumigated with EF and CO₂ in the same manner as the fruits are shown in Fig. 3, and the means of characteristics associated with phytotoxicity under gas mixture fumigation are presented in Table 4.

Vegetables with low density such as parsley could not be loaded into the fumigation box to achieve a 0.1 kg/l loading factor, so that the loading factor (kg/l) was not identical among the three vegetables. However, gas concentrations for parsley and broccoli changed substantially less than those for string beans immediately following dosing of the gas mixture of EF and CO₂.

The surface areas of both vegetables showing low concentrations are enlarged by their unique shapes. Parsley leaves are finely divided and broccoli possesses numerous small flower buds. EF gas is thus expected to be rapidly adsorbed to these vegetables. In contrast, string beans has a smooth surface and relatively low surface area. Adsorption to string bean was small, resulting in a higher change in EF gas concentration during exposure.

With respect to the quality change of fumigated vegetables, the vegetables with large surface areas, parsley and broccoli, lost weight dramatically over 7 days of storage. The surface color of treated broccoli was not inferior to that of the untreated control. Some string beans showed a slight change of color, with darkening at the tips. A marked change of color, accompanied by severe wilting.
was observed in parsley. The triangle test for flavor showed no significant difference between treated and untreated string bean or broccoli, whereas a significant (P < 0.05, chi-square test) difference was found for parsley. The distinct smell of parsley was strengthened by fumigation and the surface of leaves was coated with a viscous liquid. Stewart and Mon (1984) reported that outer leaves of lettuce appeared to be more resistant to EF injury than second and third inner leaves, though they found no loss of flavor and odor compared to controls. Thus leafy vegetables such as parsley that have a soft or tender leaf may be susceptible to EF and CO₂ fumigation; however, broccoli showed no change in quality despite its tender flower buds. Thus, the phytotoxicity of EF and CO₂ fumigation to vegetables appears to be determined not only by the physical weakness of leaves or surfaces but by other factors.

Summarizing the results described above, considered comprehensively with respect to phytotoxicity on fruits and vegetables, gas mixture fumigation with EF and CO₂ influences Satsuma mandarin by causing softening over extended storage after treatment; however, the difference between treated and untreated fruits was small and might be negligible in practice, given the absence of detectable change in flavor or color of either surface or flesh. Concerning the quality of fruits other than those we investigated, table grapes were reported to tolerate 5.0% EF treatment for 1 or 2 h, although with increased rachis browning (Simpson et al., 2007). We accordingly conclude that a gas mixture of EF and CO₂ is able to be used for quarantine treatment of fruit. In vegetables, a certain level of damages on leafy vegetables was observed in our study, but flavor damage did not appear except on parsley. Epenhuijsen et al. (2007) reported that EF had no effect on skin color or incidence of rot and produced no visual signs of phytotoxicity on onions fumigated with EF at dosages up to 324 g/m³ as Vapormate™. We propose that the use of a gas mixture of EF and CO₂ will be possible for vegetables without tender leaves. For leafy vegetables, gas mixtures containing EF must be carefully applied or limited in use, such as for vegetables destined for processing or cooking before being offered to consumers.

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和 文 摘 要

ギ酸エチル及び二酸化炭素混合ガスくん蒸の果実（バナナ、パイナップル、イチゴ、カボチャ、ブロッコリー、ミカン及びその他野菜類）に対する品質影響

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1. 新規薬剤であるギ酸エチルによる果実の品質に対する影響を調査するため、フィリピン産のバナナ及びパイナップル、佐賀県産イチゴ（品種：さちのか）、南アフリカ共和国産ブロッコリー、愛媛県産ミカン及び鹿児島県産西洋カボチャ（品種：くりやか）を用いて、ギ酸エチル75.2mg/l及び二酸化炭素374.9mg/lの混合ガス（ギ酸エチル16.7%+二酸化炭素83.3%、w/w）、15℃、3時間のくん蒸条件下でくん蒸し、重量（kg）、糖度（Brix%）、硬度（kg）、果実色（3段階評価法として、L值、a′b′値、味（3点識別法）についての影響を比較した。また、茎葉野菜類として、沖縄県産未成熟インゲン、茨城県産パセリ及び香川県産ブロッコリーについて、上記くん蒸条件のギ酸エチル及び二酸化炭素混合ガスのくん蒸で障害が発生するかどうか予備的な調査を実施した。

2. その結果、ギ酸エチルのガス濃度は、品目の表面色が大きい場合に低下する傾向を示し、くん蒸において、くん蒸の影響は吸収より大きいと考えられた。バナナ、パイナップル、イチゴ及び西洋カボチャでは、重量、硬度、果実色、糖度及び味について、バナナの果皮色と西洋カボチャの果皮色を除いて、くん蒸区とくん蒸区の差異は認められなかった。

3. バナナの果皮色のくん蒸では、くん蒸区の果実でギ酸エチルを投薬した投薬区に違いの果皮には、やけ症跡が認められる場合があったが、原因として、液体でギ酸エチルを投薬したため、気化が不十分なギ酸エチル蒸気がバナナ表面に暴露したことが考えられた。しかしながら、実際の商業くん蒸では、ギ酸エチルと二酸化炭素の混合製剤による気化投薬が実施されるため、バナナ果皮の障害を低減又は回避できる可能性があると考えられる。また、ギ酸エチルくん蒸がバナナの熱変を促促することは認められなかった。

4. 西洋カボチャでは、食味試験でくん蒸区とくん蒸区には有意差が認められなかったが、くん蒸区の果食が低下したということではなく、多くのくん蒸試験バニリスタはくん蒸区の果食が良好とコメントしており、原因として、くん蒸試験でくん蒸果果実が大さかったため、障害試験にくん蒸する果実数が限られ、個体差が評価されてしまったと考えられた。

5. グレープフルーツとミカンでは、重量、硬度、L′a′b′値空間による果実色、糖度及び味について、くん蒸7日後では、くん蒸区のミカンがやや酸化する傾向を示したものの、くん蒸区とくん蒸区の間に差異は認められなかった。

6. 茎葉野菜類では、パセリとブロッコリーをくん蒸した場合のギ酸エチル濃度は、未成熟インゲンの場合と比較して、くん蒸直後から大きく低下し、それらの重量はくん蒸後7日間で大きく減少した。さらに、パセリのくん蒸区では、果の変化及びしぶれ、強いパセリ臭及び表面の湿潤化が確認され、果食が対照区に比較して有効に劣化した。一方、ブロッコリー及び未成熟インゲンでは、くん蒸区と比べてなんら外観等に遅色がなく、果食も無処理区と差がなかった。

7. 結論として、ギ酸エチルと二酸化炭素の混合くん蒸は、生果実にほとんどの問題なく適用できると考えられ、野菜類も種類によっては、カボチャのように適用できると考えられた。しかしながら、茎葉野菜類では、ブロッコリーは問題がないが、パセリでは品質に与える影響が大きかったため、その使用には注意が必要であり、食品加工できる果実など、適用できる品目が限られると考えられた。

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