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Mechanism of Off-Flavor Production in *Brassica* Vegetables under Anaerobic Conditions

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Abstract

Mechanism of the production of off-flavor in Brassica vegetables, mainly broccoli stored under anaerobic conditions was studied. Methanethiol and dimethyl disulfide were identified as the volatile sulfur compounds in the headspace of anaerobically stored *Brassica vegetables*, including broccoli. When the activity of *C-S* lyase, a key enzyme for the formation of volatile sulfur compounds, was measured, no significant changes were observed in the *C-S* lyase activity in broccoli kept under anaerobic and aerobic conditions. The most obvious difference between anaerobically and aerobically kept broccoli was the degradation of the cell membranes. These results suggest that under anaerobic conditions volatile sulfur compounds are formed by the degradation of cellular membrane lipids and loss of intracellular compartmentation, allowing the enzyme-substrate reaction to proceed. Furthermore, the factors which affect the production of the volatile sulfur compounds, under anaerobic conditions were also investigated. Methanethiol which was one of the first compounds formed under anaerobic conditions appears to be primarily responsible for the off-flavor. Therefore, the chemical nature of the formation processes of methanethiol was also analyzed.

Discipline: Postharvest technology
Additional key words: broccoli, storage, volatile sulfur compounds

Introduction

Modifying the storage atmosphere by lowering O_2 and/or increasing CO_2 prolongs storage life and enables to preserve the high quality of many fresh fruits and vegetables. Packaging with polymeric films with specific gas permeabilities creates a modified atmosphere which exerts a beneficial effect on the extension of the storage life of various fruits and vegetables. The composition of the atmosphere in the package depends on the respiration rate of the commodity and the gas permeation rate of the film. However, when the respiration rate increases because of the increase in the holding temperature, O_2 concentration decreases and CO_2 concentration increases in the package to levels that result in the development of an off-flavor if the gas permeability of the film is insufficient.

In Japan, *Brassica* vegetables are commercially important crops, and among *Brassica* vegetables, broccoli is a highly perishable commodity and its quality and shelf-life markedly depend on the storage conditions. Modifying the atmosphere storage of broccoli under suitable O_2 and CO_2 conditions has enabled to extend the shelf-life of broccoli. However, fresh broccoli kept in modified atmosphere storage with very low O_2 and/or very high CO_2 levels produced an off-flavor, although the original appearance was retained[7]. Although the production of this offensive flavor appears to be a critical factor responsible for market losses in broccoli, the mechanism of off-flavor formation under anaerobic conditions has not been elucidated. An understanding of the mechanism(s) involved in the development of off-flavor could contribute to the improvement of the shelf-life of broccoli and the extension of its post-harvest availability. In this paper, the mechanism of the formation of undesirable flavor in *Brassica* vegetables, especially broccoli stored under anaerobic conditions is described.

Off-flavor production in *Brassica* vegetables stored under anaerobic conditions

Brassica vegetables stored under anaerobic condi-

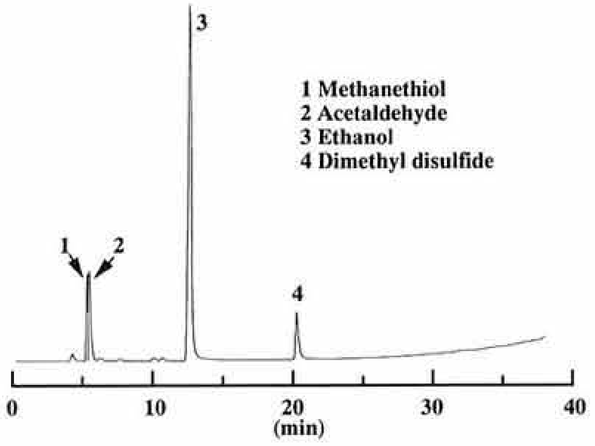

Fig. 1. GC-FID chromatogram of volatile compounds in the headspace from anaerobically stored broccoli[3)]

tions developed off-flavor. As shown in Fig. 1, ethanol, acetaldehyde, methanethiol, and dimethyl disulfide were identified as the volatile compounds in the headspace of anaerobically stored broccoli (*Brassica oleracea* L. var. *italica*). Ethanol and acetaldehyde formation may result from anaerobic respiration. Volatile sulfur compounds, such as methanethiol and dimethyl disulfide, are mainly involved in the production of the undesirable flavor of broccoli stored under anaerobic conditions. These volatile compounds were also formed in other *Brassica* vegetables, such as cabbage (*B. oleracea* L. var. *capitata*), cauliflower (*B. oleracea* L. var. *botrytis*), Chinese cabbage (*B. campestris* L. ssp. *pekinensis*) and komatsuna (*B. campestris* L.) stored under anaerobic conditions. In *Brassica* vegetables, broccoli easily developed a strong off-flavor under anaerobic conditions. Therefore, broccoli was used as a material for the following experiments.

Factors controlling the production of volatile sulfur compounds under anaerobic conditions

1) Plant parts

A number of microorganisms have been reported to produce methanethiol. However, a recent study carried out by Derbali et al.[8)] suggested that volatile sulfur compounds produced in broccoli under anaerobic conditions were of plant origin, which was confirmed by using sterile broccoli seedlings. The proposed mechanism for the formation of volatile sulfur compounds in *Brassica* vegetables is illustrated in Fig. 2. To determine the levels of volatile sulfur compounds, fresh broccoli florets were divided into flower buds and pedicels which were analyzed for the content of *S*-methyl-L-cysteine sulfoxide and the activity of *C-S* lyase. Production of methanethiol and dimethyl disulfide under anaerobic conditions was significantly higher in flower buds than in pedicels (Fig. 3), and likewise, the content of their precursor, *S*-methyl-L-cysteine sulfoxide, was about 4 times higher in flower buds (Table 1). The activity of *C-S* lyase, which catalyzes the conversion of *S*-methyl-L-cysteine sulfoxide to methanethiol and dimethyl disulfide, was significantly higher in flower buds than in pedicels (Table 1).

Table 1. Concentration of *S*-methyl-L-cysteine sulfoxide and activity of *C-S* lyase in fresh broccoli flower buds and pedicels[6)]

Plant part	*S*-Methyl-L-cysteine sulfoxide (mg/g f.w.)	*C-S* lyase activity (units/g f.w.)
Flower buds	2.12 ± 0.004	7.7 ± 0.57
Pedicels	0.51 ± 0.086	1.2 ± 0.06

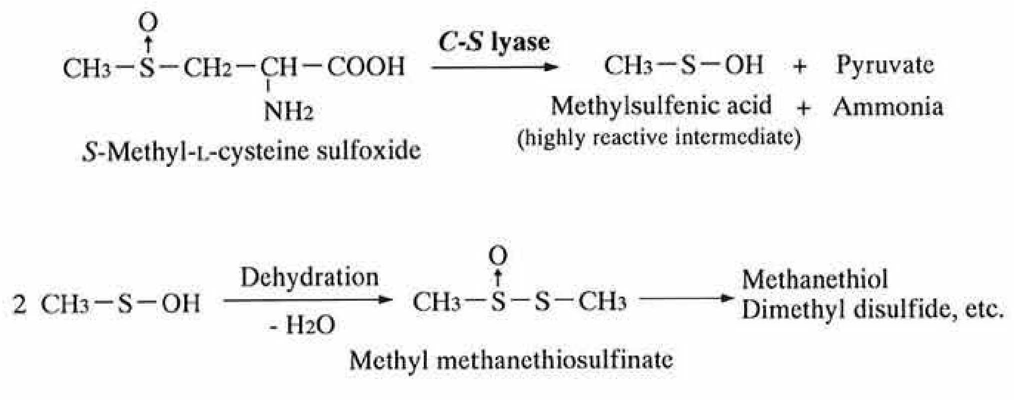

Fig. 2. Proposed mechanism for the formation of volatile sulfur compounds (Marks et al., 1992)[10)]

2) Temperature

Fresh broccoli florets were stored at 10, 20, and 30°C under anaerobic conditions. High temperature promoted the production of methanethiol and dimethyl disulfide from anaerobically treated florets (Fig. 4). It is well known that temperature is the major environmental factor in the postharvest life of fresh vegetables because, within the physiological temperature range, the velocity of a biological reaction, increases 2- to 3-fold for every 10°C rise in temperature. Therefore, the formation of methanethiol and dimethyl disulfide from anaerobically treated broccoli was affected by the temperature.

3) Effect of the duration of the low temperature pre-storage period

After fresh heads were pre-stored in the air at 1°C for 7, 14 and 21 days, the florets were kept under anaerobic conditions. Low temperature pre-storage reduced the production of volatile sulfur compounds under anaerobic conditions, even though the *S*-methyl-L-cysteine sulfoxide content and *C-S* lyase activity did not decrease[6]. It is thus assumed that the decrease in the production of volatile sulfur compounds is due to other factors, along with the increase in the duration of the pre-storage period. The visual appearance, such as green color and tightness of head, deteriorated with the increase of the duration of the period of pre-storage at low temperatures.

Mechanism of formation of volatile sulfur compounds

1) Loss of intracellular compartmentation

To analyze the mechanism of formation of vola-

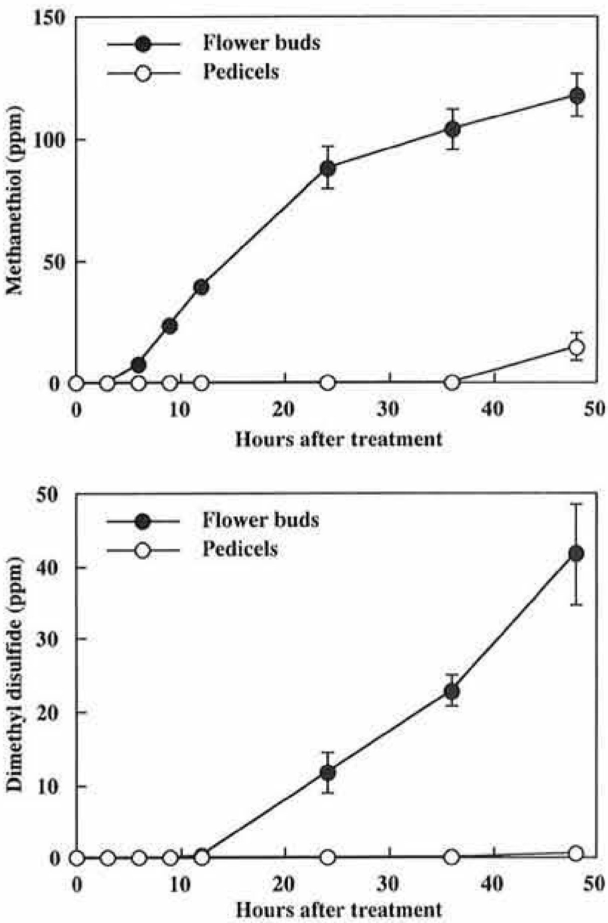

Fig. 3. Methanethiol and dimethyl disulfide production from broccoli flower buds or pedicels under anaerobic conditions
Fresh flower buds or pedicels were sealed in glass bottles with 100% N_2 and held at 20°C[6].

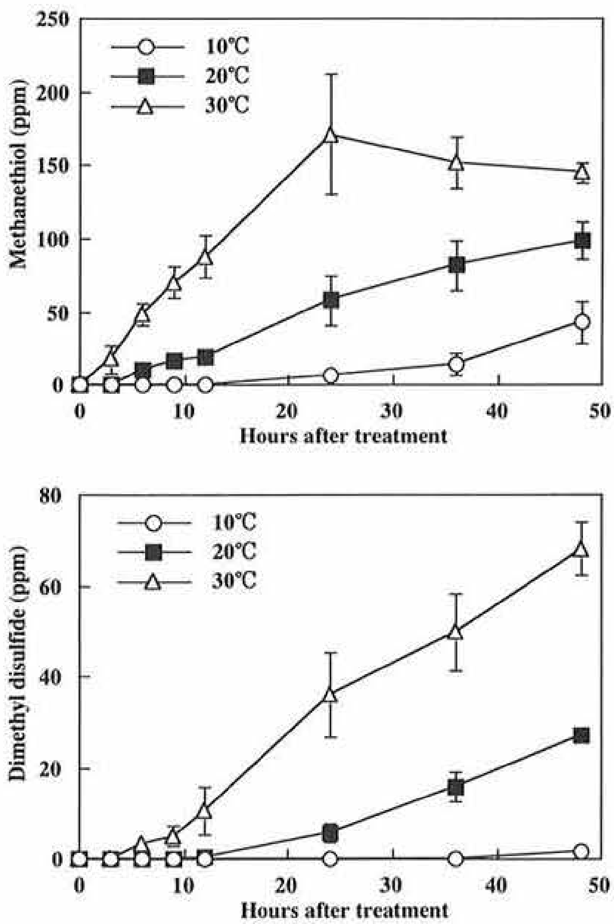

Fig. 4. Effect of holding temperature on the formation of methanethiol and dimethyl disulfide from broccoli florets under anaerobic conditions
Fresh florets were sealed in glass bottles with 100% N_2 and held at 10, 20 and 30°C[6].

tile sulfur compounds, freshly harvested broccoli were stored under anaerobic conditions. During the storage, there was no significant difference in the activity of *C-S* lyase between the anaerobically kept and aerobically kept broccoli, though a large amount of volatile sulfur compounds was generated[3]. These observations suggest that the formation of volatile sulfur compounds may not be associated with the increase in the activity of *C-S* lyase. The percentage of electrolyte leakage from flower bud sections of aerobically kept broccoli increased gradually during storage (Fig. 5), whereas that from flower bud sections of anaerobically kept broccoli increased rapidly. Furthermore, the free fatty acid to total fatty acid ratio of both aerobically and anaerobically kept broccoli increased during storage, while the ratio in anaerobically kept broccoli was significantly higher than that in aerobically kept broccoli[3] (data not shown). These results suggest that anaerobic treatment accelerated cell membrane degradation compared to natural senescence of broccoli under aerobic conditions. Significant cell membrane degradation was reflected by the large increase in the percentage of electrolyte leakage and the rise in the free fatty acid to total fatty acid ratio. Free fatty acid accumulation in the bilayered membrane has been correlated with the appearance of gel phase domains[1,11,12]. The occurrence of gel phase lipid domains in the membrane leads to a packing defect resulting in the loss of selective membrane permeability, particularly to ions and small molecules.

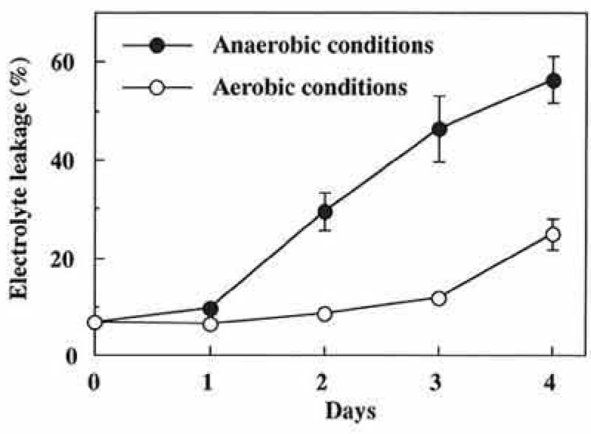

Fig. 5. Changes in percentage of electrolyte leakage from broccoli sections during storage
Flower buds were cut longitudinally into 2 parts with a razor and shaken in a flask containing deionized water. Electric conductivity of the sample medium was measured using a conductivity meter. Total electrolyte leakage was obtained by boiling samples[3].

Presumably, the loss of membrane integrity contributes to the loss of intracellular compartmentation.

Much of the characteristic odor and flavor associated with *Brassica* vegetables is due to the degradation of non-protein sulfur-containing amino acids, such as *S*-methyl-L-cysteine sulfoxide by *C-S* lyase in the tissues without reaction with the substrate until the tissues are physiologically and/or physically damaged.

These results indicate that, under anaerobic conditions, volatile sulfur compounds, such as methanethiol and dimethyl disulfide are formed by the degradation of cellular membrane lipids and the loss of intracellular compartmentation, thus, allowing enzymes and their substrates to react.

2) Methanethiol production

Methanethiol which was one of the first compounds formed under anaerobic conditions appears to be primarily responsible for the offensive odor. Dimethyl disulfide may evolve from the oxidation of methanethiol. However, the chemical nature of the formation processes of methanethiol in *Brassica* has not been well-characterized. In this study, broccoli tissues were mechanically injured in order to disrupt intracellular compartmentation and the factors responsible for the formation of methanethiol from *S*-methyl-L-cysteine sulfoxide were investigated.

A solution of disrupted fresh broccoli tissues was mixed with one of the reagents. Addition of L-methionine, L-cystine or oxidized glutathione to the solution did not induce the formation of methanethiol, whereas the addition of L-cysteine or reduced glutathione to the solution resulted in the production of a considerable amount of methanethiol and small amounts of some other unknown volatile sulfur compounds (Table 2). Reducing agents (L-ascorbic acid or sodium borohydride), however, did not induce the production of more methanethiol, which indicates that free SH of L-cysteine or reduced glutathione is responsible for the formation of methanethiol.

The fresh tissue solution was passed through a filter with a molecular weight cut off of 10,000. The filtrate was mixed with L-cysteine. A significant amount of methanethiol evolved after the addition of L-cysteine (Table 3), suggesting that the reaction leading to the formation of methanethiol by the addition of L-cysteine, may not be enzymatic. However, the same tests with a tissue solution prepared from previously autoclaved broccoli failed to reveal the production of methanethiol, which indi-

Table 2. Effects of sulfur-containing amino acids, glutathione, L-ascorbic acid, and sodium borohydride on the formation of methanethiol in the headspace of stoppered test tubes containing a disrupted tissue solution of fresh broccoli[4)]

Treatment	Methanethiol (peak area × 10^{-4})
Control	0.7
L-Cysteine	131.4
L-Methionine	0.7
L-Cystine	0.6
Glutathione (reduced form)	91.1
Glutathione (oxidized form)	0.8
L-Ascorbic acid	0.7
Sodium borohydride	0.8

The reaction mixture with each 10 mM reagent except for the control was incubated at 20°C for 1 h.

Table 3. Formation of methanethiol from disrupted tissue solution of broccoli[4)]

Treatment	Addition of L-cysteine[a)] (mM)	Methanethiol (peak area × 10^{-4})
Disrupted fresh tissue solution	No 5	0.7 108.7
Disrupted fresh tissue solution (Mol. wt. ≦10,000 fraction)	No 5	1.3 119.4
Disrupted tissue solution prepared from heat-treated tissues	No 5	0.6 1.6

The reaction mixture was incubated at 20°C for 1 h.
a): Final concentration in the reaction mixture.

Table 4. Methyl methanethiosulfinate formation in the disrupted tissue solution of broccoli after each treatment[4)]

Treatment	Methyl methanethiosulfinate (peak area × 10^{-4})
Disrupted fresh tissue solution (initial)	5.2
Disrupted fresh tissue solution incubated at 20°C for 2 h	58.9
Disrupted fresh tissue solution + 10 mM *S*-methyl-L-cysteine sulfoxide[a)] incubated at 20°C for 2h	229.9
Disrupted fresh tissue solution + 5 mM L-cysteine[a)] incubated at 20°C for 2 h	Not detected
Disrupted tissue solution prepared from tissues exposed to 100°C for 10 min	Not detected

a): Final concentration in the reaction mixture.

cates that enzyme(s) were involved in some steps of the pathway of methanethiol formation.

Methanethiol is considered to be produced as a result of the conversion of *S*-methyl-L-cysteine sulfoxide to an unstable methanesulfenic acid intermediate by *C-S* lyase in *Brassica* which is then condensed and dehydrated to form more stable methyl methanethiosulfinate. The amount of methyl methanethiosulfinate which was detected in the solution of disrupted fresh tissues immediately after preparation (Table 4), increased about 10 times during a 2-h incubation at 20°C. The reaction mixture of the solution of disrupted fresh tissues and *S*-methyl-L-cysteine sulfoxide released a large amount of methyl methanethiosulfinate. When L-cysteine was mixed with the solution of disrupted fresh tissues, methyl methanethiosulfinate disappeared. No methyl methanethiosulfinate was detected in the disrupted tissue solution, prepared from heat-treated broccoli. The thiolsulfinates, -SO-S-, react rapidly with L-cysteine[2)]. The following reaction might have occurred in the mixture solution of methyl methanethiosulfinate and L-cysteine.

$$CH_3\text{-}SO\text{-}S\text{-}CH_3 + HS\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH \rightarrow CH_3\text{-}SH + CH_3\text{-}SO\text{-}S\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$$

In our study, only a trace amount of methanethiol was produced after fresh tissue disruption, and almost concurrently with methyl methanethiosulfinate. Derbali et al.[8)] and Di Pentima et al.[9)] reported that a considerable increase in the content of free amino acids, particularly sulfur-containing amino acids and their derivatives, was observed in broccoli stored under anaerobic conditions, which resulted in the production of volatile sulfur compounds including methanethiol. Their findings suggest that methanethiol may be formed as a result of the reaction of methanethiosulfinate with free SH-containing amino acids and peptides under anaerobic conditions.

Prevention of off-flavor production

Methanethiol and dimethyl disulfide are derived from a common precursor, methyl methanethiosulfinate, which is a product of the *C-S* lyase-catalyzed degradation of *S*-methyl-L-cysteine sulfoxide. The selection of or breeding for cultivars with low *S*-methyl-L-cysteine sulfoxide content or *C-S* lyase activity may enable to develop broccoli with fewer undesirable volatile sulfur compounds under anaerobic conditions. Presently, there are no broccoli cultivars with an extremely low production of volatile sulfur compounds under anaerobic conditions[5]. Moreover, in common to *Brassica* vegetables, the production of volatile sulfur compounds is significantly higher in flower buds than in leaves or stems[5]. These findings suggest that the off-flavor is readily generated in broccoli compared with other *Brassica* vegetables, because the main edible portion of broccoli consists of flower buds. Therefore, proper storage and distribution management is presumably the major condition for postharvest handling of *Brassica* vegetables, especially broccoli.

References

1) Barber, R. F. & Thompson, J. E. (1983): Neutral lipids rigidify unsaturated acyl chains in senescing membranes. *J. Exp. Bot.*, **34**, 268-276.
2) Cavallito, C. J., Buck, J. S. & Suter, C. M. (1944): Allicin, the antibacterial principle of *Allium sativum*. II. Determination of the chemical structure. *J. Am. Chem. Soc.*, **66**, 1952-1954.
3) Dan, K. et al. (1997): Formation of volatile sulfur compounds in broccoli stored under anaerobic condition. *J. Jpn. Soc. Hort. Sci.*, **65**, 867-875.
4) Dan, K., Nagata, M. & Yamashita, I. (1997): Methanethiol formation in disrupted tissue solution of fresh broccoli. *J. Jpn. Soc. Hort. Sci.*, **66**, 621-627.
5) Dan, K., Nagata, M. & Yamashita, I. (1997): *S*-methyl-L-cysteine sulfoxide content, *C-S* lyase activity and methanethiol production in broccoli cultivars. *J. Jpn. Soc. Hort. Sci.*, **66** (suppl. 2), 78-79 [In Japanese].
6) Dan, K., Nagata, M. & Yamashita, I. (1998): Effects of pre-storage duration and storage temperatures on the formation of volatile sulfur compounds in broccoli under anaerobic conditions. *J. Jpn. Soc. Hort. Sci.*, **67**, 544-548.
7) Dan, K., Nagata, M. & Yamashita, I. (1998): Volatile sulfur compounds production and visual quality of broccoli. *J. Jpn. Soc. Hort. Sci.*, **67** (suppl. 1), 304.
8) Derbali, E., Makhlouf, J. & Vezina, L.-P. (1998): Biosynthesis of sulfur volatile compounds in broccoli seedlings stored under anaerobic conditions. *Postharv. Biol. Technol.*, **13**, 191-204.
9) Di Pentima, J. H. et al. (1995): Biogenesis of off-odor in broccoli storage under low-oxygen. *J. Agric. Food Chem.*, **43**, 1310-1313.
10) Marks, H. S. et al. (1992): *S*-Methylcysteine sulfoxide in *Brassica* vegetables and formation of methyl methanethiosulfinate from Brussels sprouts. *J. Agric. Food Chem.*, **40**, 2098-2101.
11) McKersie, B. D., Crowe, J. H. & Crow, L. M. (1989): Free fatty acid effects on leakage, phase properties and fusion of fully hydrated model membranes. *Biochim. Biophys. Acta*, **982**, 156-160.
12) Yao, K., Paliyath, G. & Thompson, J. E. (1991): Nonsedimentable microvesicles from senescing bean cotyledons contain gel phase-forming phospholipid degradation products. *Plant Physiol.*, **97**, 502-508.

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