

# Photosensitized Oxidative Reaction of 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone

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2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) in absolute alcohol containing chlorophyll was exposed to light while being bubbled with oxygen. The photooxidation products, including ethyl pyruvate, ethyl lactate, acetic acid, ethyl 2-acetoxypropionate, acetoxyacetone, 1,2-ethanediol, acetoxy-2,3-butanedione, 2-oxopropyl 2-acetoxypropionate, lactic acid, and 2-acetoxypropionic acid were identified using gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). Possible formation mechanisms for these products are postulated. The antiphotooxidative effect of capsanthin and lutein on DMHF in absolute alcohol containing chlorophyll was studied under light exposure while bubbling with oxygen. Both capsanthin and lutein showed the antiphotooxidative effect on DMHF, and their antiphotooxidative activity increased with increasing concentration. The activity of capsanthin, which contains 11 conjugated double bonds, a conjugated keto group, and one cyclopentane ring, was higher than that of lutein, which contains 10 conjugated double bonds but neither a conjugated keto group nor a cyclopentane ring.

**Keywords:** 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone; photosensitized oxidation; photooxidation; capsanthin; lutein

## INTRODUCTION

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone or DMHF, which has been described as possessing a caramel-like, sweet, fruity, and burnt pineapple-like flavor (Pittet et al., 1970; Rodin et al., 1965), has been extensively used for flavoring of jams, jellies, beverages, ice creams, alcoholic drinks, and sweets (Hirvi et al., 1980).

DMHF has been found in fruits, heat-processed foods, and some model system reactions, as shown in Table 1. In fruits, a glucoside of DMHF has been identified (Wu et al., 1990, 1991; Mayerl et al., 1989; Krammer et al., 1994). It seems that a biogenetic pathway is involved in DMHF formation in fruits. DMHF is also found in heat-processed foods and model system reactions, indicating that a thermal reaction of sugar or its derivatives could generate this compound.

It has been reported that DMHF is unstable in air and in aqueous solutions (Hodge et al., 1963). The optimal stability of DMHF is pH 4 at 20 °C (Hirvi et al., 1980); while at a roasting temperature of 160 °C, the stability of DMHF increased with increasing pH (Shu et al., 1985). Because no information exists about the photooxidative stability of DMHF, the purpose of this study was to investigate the stability of DMHF in absolute alcohol containing chlorophyll, a photosensitizer, under light exposure while bubbling with oxygen. The photooxidation products from DMHF were also investigated.

Our previous study reported that two carotenoids, capsanthin and lutein, exhibited the antiphotooxidative effect on soybean oil as well as some flavor compounds (Chen et al., 1996). The effect of capsanthin and lutein on the photosensitized oxidation of DMHF was also reported in this study.

**Table 1. Sources of 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone**

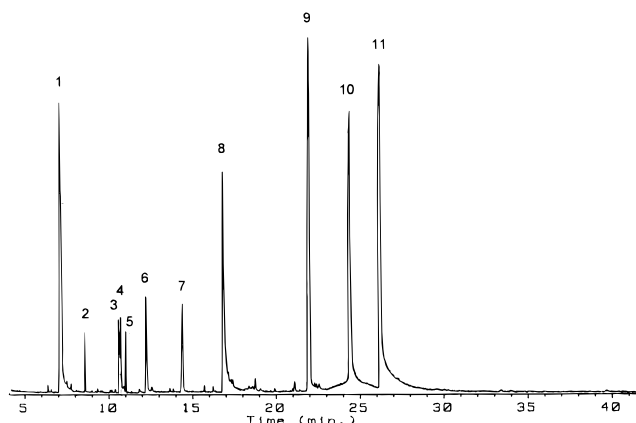
source	ref
from fruits or fruit juice	
pineapple	Rodin et al., 1965
strawberry	Re et al., 1973; Pickenhagen et al., 1981
raspberry	Honkanen et al., 1980
mango	Pickenhagen et al., 1981
blackberry	Sen et al., 1991
grapefruit juice	Lee and Nagy, 1987
tomato	Buttery et al., 1994
canned orange juice	Tatum et al., 1975
arctic bramble	Kallio, 1976
from heat-processed foods	
beef broth	Tonsbeek et al., 1968
roasted almonds	Takei and Yamanishi, 1974
roasted coffee	Tressl et al., 1978
sponge cake	Takei, 1977
wheat bread crust	Schieberle, 1990
popcorn	Schieberle, 1991
from model system reaction	
thermal degradation of fructose	Shaw et al., 1968
thermal degradation of glucose	Fagerson, 1969; Heyns et al., 1966
thermal degradation of rhamnose	Schieberle, 1992
thermal degradation of 1-deoxy-1-piperidinofructose	Mills et al., 1969
thermal degradation of 1-deoxy-1-prolinofructose	Mills and Hodge, 1976
thermal degradation of fructose-1,6-diphosphate	Schieberle, 1992
thermal reaction of alanine/rhamnose	Shaw and Berry, 1977
thermal reaction of glucose/arginine/egg lecithin	Takei, 1977
thermal reaction of cysteine/glucose	Eiserich et al., 1992
thermal reaction of histidine/glucose	Gi and Baltes, 1995

## EXPERIMENTAL PROCEDURES

**Materials.** Standards of DMHF, ethyl pyruvate, ethyl lactate, acetic acid, lactic acid, and 2-acetoxypropionic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI). Capsanthin and lutein were obtained from Kalsec Co. (Kalam-

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**Figure 1.** GC chromatogram of the photosensitized oxidation products from DMHF. Conditions: column, DB-WAX fused silica capillary (60 mm  $\times$  0.32 mm i.d.); temperature, 50–195  $^{\circ}$ C, programmed at 2  $^{\circ}$ C/min; detector, flame ionization.

azoo, MI). Chlorophyll was extracted and purified from spinach according to the method of Omata and Murata (1980). Absolute alcohol was obtained from Pharmco Products Co. (Brookfield, CT).

**Photosensitized Oxidation of DMHF.** A sample of  $1 \times 10^{-2}$  M DMHF in 100 mL of absolute alcohol containing 10 ppm chlorophyll was prepared in a 250 mL flask. The photosensitized oxidation of DMHF was carried out under light exposure while bubbling with oxygen at 260 mL/min for 2 h at 20  $^{\circ}$ C according to the method of Chen and Ho (1996). The light intensity at the sample position was 67 000 lux. The reaction solution was then concentrated and analyzed by gas chromatography and gas chromatography–mass spectrometry (GC-MS). A Hewlett-Packard (H/P) Model 5880A gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (DB-WAX, J & W Scientific, 60 m  $\times$  0.32 mm i.d.) was used in this study. The GC oven was temperature programmed from 50 to 195  $^{\circ}$ C at a rate of 2  $^{\circ}$ C/min. The carrier gas (He) flow rate was 27 cm/s. For GC-MS analysis, a Kratos MS-30 mass spectrometer and an H/P Model 5880 GC unit equipped with the same column described above were used. Both electron ionization (EI) and chemical ionization (CI) methods were used in this study. Isobutyl alcohol was the reagent gas for the CI method.

**Synthesis of Ethyl 2-Acetoxypropionate.** In a reaction flask equipped with a thermometer and a condenser, ethyl lactate (0.086 mol), acetic acid (0.083 mol), and 4 drops of sulfuric acid were mixed with a stirring bar. The mixture was heated and refluxed at 118  $^{\circ}$ C for 1 h and then cooled to room temperature. The reaction mass was diluted with 20 mL of diethyl ether and washed with saturated salt water until neutral, and then the ether layer was dried and concentrated. The product was 95% ethyl 2-acetoxypropionate based on GC analysis.

**Antioxidative Effect of Capsanthin and Lutein on the Photosensitized Oxidation of DMHF.** A mixture of  $1 \times 10^{-3}$  M DMHF in 100 mL of absolute alcohol containing 10 ppm chlorophyll and either  $1.0 \times 10^{-5}$  or  $2.5 \times 10^{-5}$  M capsanthin or lutein was prepared in a 250 mL flask. Each mixture was then exposed to light while being bubbled with oxygen at 20  $^{\circ}$ C as described above. The antioxidative effect of capsanthin and lutein was determined by using a Hitachi U-3110 spectrophotometer (Danbury, CT) in which the percentage of substrate remaining was calculated by measuring the decrease of optimum wavelength absorbance (290 nm) of DMHF.

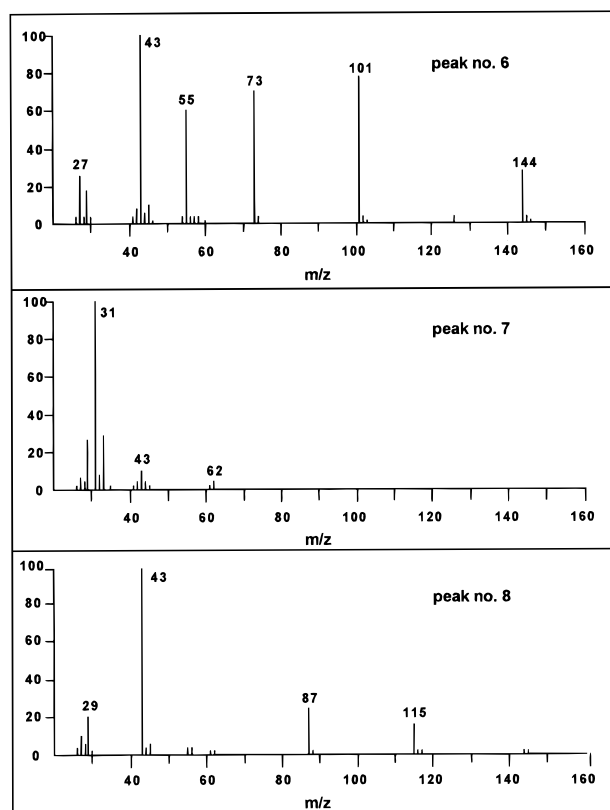
## RESULTS AND DISCUSSION

**Photosensitized Oxidation of DMHF.** After light exposure while being bubbled with oxygen for 2 h, the DMHF solution was concentrated and then analyzed with GC and GC-MS. The GC chromatogram of the photooxidation products is shown in Figure 1. In total,

**Table 2. Components Identified from the Photooxidation of DMHF at 20  $^{\circ}$ C**

peak no.	compd identified	identification
1	ethyl pyruvate	a, b
2	ethyl lactate	a, b
3	acetic acid	a, b
4	ethyl 2-acetoxypropionate	c
5	acetoxyacetone	b
6	acetoxy-2,3-butanedione	d
7	1,2-ethanediol	d
8	2-oxopropyl 2-acetoxypropionate	d
9	DMHF <sup>e</sup>	
10	lactic acid	a, b
11	2-acetoxypropionic acid	a

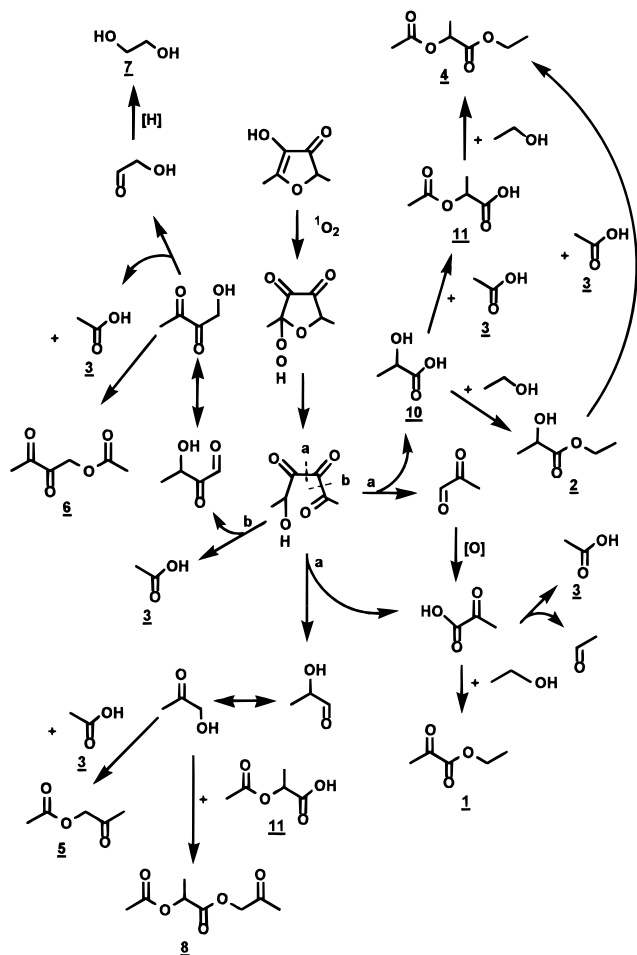
<sup>a</sup> GC analysis of authentic compounds. <sup>b</sup> Mass spectral data from Ten Noever de Brauw et al. (1983). <sup>c</sup> GC analysis of the compound we synthesized. <sup>d</sup> Tentative identification according to mass spectral data of EI and CI. <sup>e</sup> Starting material.



**Figure 2.** EI mass spectra of peaks 6, 7, and 8, DB-WAX column.

there were 11 photooxidation products. Table 2 lists these compounds which were tentatively identified by comparing their retention times and mass spectral data with those of the standards (peaks 1, 2, 3, 10, and 11) and the compound we synthesized (peak 4), by comparing the mass spectral data with the reference (peak 5), or by interpreting the EI and CI mass spectra (peaks 6, 7, and 8).

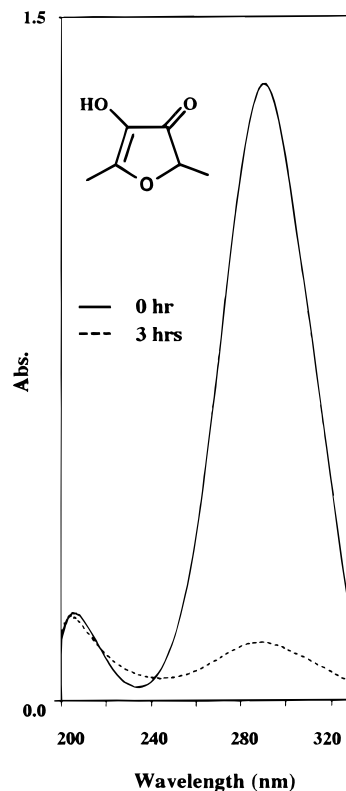
According to the CI mass spectra, the molecule weight of peaks 6, 7, and 8 were 144, 62, and 188, respectively (data not shown). The EI mass spectra of peaks 6, 7, and 8 are presented in Figure 2. In the spectrum of peak 6, the large fragments of  $m/z$  101 and 73 suggest that a stepwise cleavage of  $\text{CH}_3\text{CO}$  ( $M - 43$ ) followed by loss of CO ( $m/z$  101  $\rightarrow$   $m/z$  73) occurred. The strong fragment of  $m/z$  43 indicates the presence of an acetyl group in this structure. Based on the spectral data of EI and CI, the structure of peak 6 was proposed as acetoxy-2,3-butanedione. The strong fragment of  $m/z$



**Figure 3.** Postulated mechanism for the formation of the photosensitized oxidation products from DMHF in the absolute alcohol containing 10 ppm chlorophyll at 20 °C.

31 in the spectrum of peak 7 suggests the presence of a  $-\text{CH}_2\text{OH}$  group. The loss of mass 31 ( $M - 31$ ) suggests this mass could be  $\text{HOCH}_2$ . Therefore, the structure of peak 7 was proposed as 1,2-ethanediol. In the spectrum of peak 8, the fragments of  $m/z$  115 and 87 indicates cleavage of  $\text{CH}_3\text{COCH}_2\text{O}$  ( $M - 73$ ) followed by loss of  $\text{CO}$  ( $m/z$  115  $\rightarrow$   $m/z$  87). The strong fragment of  $m/z$  43 suggests the presence of an acetyl group in this structure. Combining all of the information, peak 8 was proposed as 2-oxopropyl 2-acetoxypropionate.

The possible mechanism for the formation of these photooxidation products from DMHF is summarized in Figure 3. The singlet oxygen ( $^1\text{O}_2$ ) is generated under the light exposure in the presence of chlorophyll as a photosensitizer and oxygen (Foote, 1979). This  $^1\text{O}_2$ , which is a very reactive form of oxygen, attacks the double bond at the C-5 position to generate the hydroperoxide. The ring of this hydroperoxide is opened and forms a 5-hydroxy-2,3,4-hexanetrione intermediate, which is then hydrolyzed from either position a or position b to form the primary products or intermediates including lactic acid (peak 10), pyruvic aldehyde, pyruvic acid, lactic aldehyde, acetic acid (peak 3), and acetoaldehyde. The lactic acid then undergoes a series of esterification processes with acetic acid or alcohol to form the secondary products including 2-acetoxypropionic acid (peak 11), ethyl lactate (peak 2), and ethyl 2-acetoxypropionate (peak 4). The pyruvic acid, which is a very unstable intermediate, easily undergoes an esterification reaction with ethanol to form ethyl pyruvate (peak 1) and proceeds through a degradation reaction to



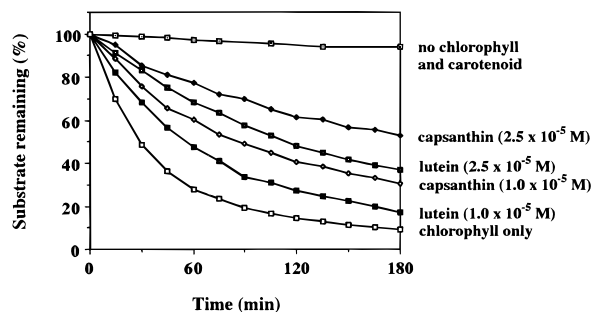
**Figure 4.** UV spectra of DMHF in the absolute alcohol after photosensitized oxidation at 20 °C for 0 and 3 h.

generate the acetic acid (peak 3). We confirmed that the ethyl pyruvate and acetic acid were the two major photooxidation products from pyruvic acid by GC analysis of photooxidation of pyruvic acid standard (data not shown). The intermediates, lactic aldehyde and acetoaldehyde, undergo a keto-enol tautomerization process to form keto alcohols, which are able to react with acid to form esters including 2-oxopropyl 2-acetoxypropionate (peak 8), acetoxyacetone (peak 5), and acetoxy-2,3-butanedione (peak 6). 1,2-Ethanediol (peak 7) could be generated from the degradation of 1-hydroxy-2,3-butanedione.

This study shows that during the photosensitized reaction DMHF degrades to primary products, which then react with alcohols or acids to form acyclic esters.

**Antioxidative Effect of Capsanthin and Lutein on the Photosensitized Oxidation of DMHF.** The effect of  $1.0 \times 10^{-5}$  or  $2.5 \times 10^{-5}$  M carotenoids including capsanthin and lutein on the photooxidative stability of DMHF in absolute alcohol containing chlorophyll was conducted under light exposure while bubbling with oxygen for 3 h. The percentage of substrate remaining was determined by spectrophotometry and was used as an index of antiphototoxidative activity of carotenoids.

The UV spectrum of DMHF after a 0 or 3 h photosensitized reaction is shown in Figure 4. The absorbance at  $\lambda_{\text{max}}$  (290nm) decreased significantly after 3 h. The percentage of DMHF remaining was calculated by measuring the decrease of the absorbance at 290 nm. Figure 5 presents the antiphototoxidative effect of capsanthin and lutein on DMHF. The more the percentage of DMHF remaining, the higher the antiphototoxidative activity they possess. The control mixture containing neither chlorophyll nor carotenoids showed only a very slight decrease in DMHF after 3 h exposure to oxygen. When chlorophyll was spiked in this solution, the percentage of DMHF decreased rapidly. This result indi-



**Figure 5.** Effect of capsanthin and lutein on the photosensitized oxidation of DMHF in the absolute alcohol containing 10 ppm chlorophyll at 20 °C.

cates that chlorophyll is a prooxidant, which is able to accelerate the singlet oxygen reaction (Bradley and Min, 1992). Both capsanthin and lutein showed the antiphototoxidative effect on DMHF, and their antiphototoxidative activity increased when their concentrations increased from  $1.0 \times 10^{-5}$  to  $2.5 \times 10^{-5}$  M as shown in Figure 5. On comparison of reactions containing the same concentrations of capsanthin and lutein, the antiphototoxidative activity of capsanthin, which contains 11 conjugated double bonds, a conjugated keto group, and one cyclopentane ring, was higher than that of lutein, which contains 10 conjugated double bonds but neither a conjugated keto group nor a cyclopentane ring. This is the same result as found in our previous work (Chen et al., 1996). This result confirms that the antiphototoxidative activity or singlet oxygen quenching ability of carotenoids is dependent on the number of conjugated double bonds, and the conjugated keto group and cyclopentane ring in the structure of carotenoids stimulate this ability (Hirayama et al., 1994).

It has been known that carotenoids can quench singlet oxygen to reduce the photosensitized oxidation of both soybean oil and some flavor compounds, including 2-ethylfuran and 2,4,5-trimethylxazole (Lee and Min, 1990; Jung and Min, 1990; Chen et al., 1996). This study suggests that two carotenoids, capsanthin and lutein, may also be applied to food systems which contain DMHF or other flavor compounds to prevent flavor loss or minimize off-flavor formation.

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