



Oxidative stress-mediated antiproliferative effects of furan-containing sulfur flavors in human leukemia Jurkat cells



Gong-Liang Zhang, Ying Liang, Jun-Ya Zhu, Qiong Jia, Wei-Qi Gan, Li-Ming Sun, Hong-Man Hou *

School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, PR China

ARTICLE INFO

Article history:

Received 15 October 2014

Received in revised form 5 January 2015

Accepted 26 January 2015

Available online 31 January 2015

Keywords:

Sulfur flavors

Furan

Antiproliferative effect

Apoptosis

Oxidative stress

Jurkat cells

ABSTRACT

Antiproliferative effects of 15 sulfides were investigated in human leukemia Jurkat cells. Treatment with 5–50 μM of nine monosulfides and two linear disulfides did not induce DNA fragmentation. Whereas, furan-containing sulfur flavors including methyl 2-methyl-3-furyl disulfide (MMFDS), bis (2-methyl-3-furyl) disulfide (BMFDS), methyl furfuryl disulfide (MFDS) and difurfuryl disulfide (DFDS) induced DNA fragmentation to a varying extent in Jurkat cells. The cell viability-reduction effect of these sulfur flavors was in the following order: DFDS > BMFDS > MMFDS > MFDS based on the IC_{50} values. MMFDS and BMFDS, but not DFDS, significantly increased the intracellular ROS level by 1.90- and 3.02-fold, respectively. Addition of *N*-acetylcysteine (NAC) or glutathione (GSH) partially suppressed induction of DNA fragmentation, apoptosis and caspase-3 activation by MMFDS and BMFDS. These results suggest that the furan-containing disulfides have a strong antiproliferative effect, and the oxidative stress and subsequent caspase-3 activation are involved in antiproliferative effect induced by MMFDS and BMFDS in Jurkat cells.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Sulfur-containing flavor compounds have become a very important class of flavors (Sun, Tian, Zheng, Xie, & Liu, 2007). The number of sulfur-containing flavor compounds has increased quickly in the 21st century and many new sulfur-containing chemicals have been given 'generally regarded as safe' (GRAS) status by the Flavor and Extract Manufacturers Association (FEMA). The number of sulfur-containing compounds among GRAS substances announced by the FEMA has reached more than 300. Sulfur compounds contribute enzymatically-derived flavors in the *Allium* species (garlic, onion, chive) or *Cruciform* families (brussels sprouts, broccoli, cabbage, cauliflower), and thermally-generated flavors such as roasted meat, chicken, seafood, and coffee (McGorin, 2011). Due to the importance of sulfur-containing compounds for the various aromas

they produce, these compounds are widely used in the food industry. There has been a great effort directed towards the investigation of the sulfur-containing components responsible for flavor development and towards identification and characterization the sulfur-containing flavors (Kubec, Krejčová, Mansur, & García, 2013; Liu, Chen, Yin, & Sun, 2010; Mouhib, Van, & Stahl, 2013; Perez-Cacho, Mahattanatawee, Smoot, & Rouseff, 2007; Ruther & Baltes, 1994; Tian, Sun, & Huang, 2006). However, knowledge about biological function of sulfur-containing flavor compounds has not yet been fully investigated.

It has been reported that more than 30% of human cancers could be prevented by an alternative strategy of appropriate dietary modification (Willett, 2002). As anticancer drug therapies are mostly toxic, immune-suppressive, mutagenic, and even carcinogenic (McWhinney, Goldberg, & McLeod, 2009; Minami, Matsumoto, & Horiuchi, 2010), the search for natural products with chemopreventive properties and without deleterious effects is necessary. In recent years, numerous dietary natural compounds that have antiproliferative activities in tumor cells have been discovered, such as saponins and flavonoids from black bean, water-soluble enzymatic extract from rice bran, wheat flour and neferine (Guajardo-Flores, Gutiérrez-Urbe, & Serna-Saldívar, 2013; Lv et al., 2012; Poornima, Quency, & Padma, 2013; Revilla et al., 2013).

Abbreviations: GRAS, generally regarded as safe; FEMA, Flavor and Extract Manufacturers Association; PBS (–), phosphate-buffered saline without Ca^{2+} and Mg^{2+} ; $\text{H}_2\text{DCF-DA}$, 2',7'-dichlorodihydrofluorescein diacetate; OSCs, organosulfur compounds; ROS, reactive oxygen species; MMFDS, methyl 2-methyl-3-furyl disulfide; BMFDS, bis (2-methyl-3-furyl) disulfide; MFDS, methyl furfuryl disulfide; DFDS, difurfuryl disulfide; NAC, *N*-acetylcysteine; GSH, glutathione; DMTS, dimethyl trisulfide; DMTTS, dimethyl tetrasulfide; DADS, diallyl disulfide; DATS, diallyl trisulfide.

* Corresponding author. Tel./fax: +86 411 86322020.

E-mail address: hongman2011@hotmail.com (H.-M. Hou).

Epidemiological studies have provided some convincing evidence that increased dietary consumption of garlic reduces the risk of cancer (Milner, 2001). Anticancer effects of garlic are generally attributed to organosulfur compounds (OSCs) formed during garlic storage and processing, especially to allyl sulfides (Agarwal, 1996). Numerous *in vitro* studies have shown antiproliferative and apoptotic effects of allyl sulfides on cancer cells (Le Bon & Siess, 2000; Thomson & Ali, 2003). The intracellular redox environment has been suggested to modulate several cellular processes such as cell proliferation, apoptosis and survival (Ozben, 2007; Thomas & Paul, 1994; Wang, Martindale, Liu, & Holbrook, 1998). Oxidative stress induces activation of caspases, a family of cysteine proteases that are involved in induction of apoptosis (Polverino & Patterson, 1997). Various leukemia cell lines are often used to elucidate mechanism of apoptosis since anti-cancer drugs or cytotoxic reagents are more effective in leukemia than in other cancer diseases (Buttke & Sandstrom, 1994). Our previous studies have indicated that dimethyl sulfur compounds, including dimethyl trisulfide (DMTS) and dimethyl tetrasulfide (DMTTS) possessed antiproliferative effect in human leukemia cells *via* reactive oxygen species (ROS) production and a caspase-3 activation pathway (Zhang et al., 2008).

Based on the common group and structural unit, sulfur-containing flavor compounds can be classified into thiols, thiophenols, sulfides, thiocarboxylates, thioacetals, etc (Sun et al., 2007). In the present study, we selected fifteen common sulfides commercially used in food industry with a wide range of flavors, such as garlic, onion, cabbage odors, violet leaf and flowerlike aromas. To provide a better understanding of the biological function of sulfur-containing flavor compounds, we examined the effects of fifteen common sulfides on viability and induction of apoptosis of human leukemia Jurkat cells. Moreover, in order to clarify involvement of ROS production in the apoptosis, effects of antioxidants, *N*-acetylcysteine (NAC) and glutathione (GSH) on sulfide-induced apoptosis and caspase-3 activation were also examined.

2. Materials and methods

2.1. Materials and chemicals

Fifteen sulfur flavors, each with a purity of 95–99%, NAC, GSH, 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF-DA) and a caspase 3 assay kit were purchased from Sigma–Aldrich (St. Louis, MO, USA). The structures of sulfur flavors are shown in Table 1. RPMI-1640 medium and fetal bovine serum was purchased from Gibco-Invitrogen (Carlsbad, CA). Cell Counting Kit-8 solution was purchased from Dojindo Laboratories (Kumamoto, Japan). Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit was purchased from invitrogen (Carlsbad, CA, USA). All other chemicals and reagents used in this study were of analytical grade.

2.2. Cell culture and treatment

Human leukemia T cells (Jurkat cells) were cultured in RPMI-1640 medium supplemented with 10% (v/v) FBS, 50 U/ml of penicillin and 50 µg/ml of streptomycin at 37 °C under 5% CO₂ and 95% air.

All sulfur flavors were dissolved in ethanol. For experiments, cells were subcultured in complete medium and treated with each sulfur flavor at an indicated concentration (5–50 µM) or vehicle (0.1% ethanol, v/v). For antioxidant experiments, cells were pre-treated with NAC or GSH at a concentration of 1 mM for 30 min before sulfide treatment to investigate DNA fragmentation and apoptosis.

2.3. DNA fragmentation

After treatment, the cells were harvested and washed twice with ice-cold PBS (–), and were then lysed in 100 µl of hypotonic buffer containing 10 mM Tris–HCl (pH 7.4), 10 mM EDTA and 0.5% Triton X-100 on ice for 10 min. The cell lysate was centrifuged at 12,000×g for 20 min at 4 °C. The supernatant was treated with RNase at 37 °C for 1 h and then treated with proteinase K at 37 °C for 1 h. DNA was precipitated by adding NaCl and isopropanol. The DNA was dissolved in TE buffer and was analyzed on 2% agarose gel. The gel was stained with GelRed (Biotium, Inc., Hayward, CA, USA), and documented using GelCapture software (DNR Bio-Imaging Systems, Jerusalem, Israel).

2.4. Determination of cell viability

Cell viability was evaluated using a colorimetric assay with the tetrazolium salt WST-8 (Cell Counting Kit 8, CCK-8). Cells were treated with each sulfide in a 96-well plate, and incubated at 37 °C under 5% CO₂ for 24 h. After incubation, 10 µl of WST-8 was added to each well and then plates were incubated at 37 °C under 5% CO₂ for 2 h. Cell viability was calculated according to the absorbance at 450 nm in a multimode microplate reader (Infinite M200; Tecan Group Ltd., Switzerland).

2.5. Determination of intracellular ROS production

The reagent 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF-DA) was used as an intracellular fluorescence probe to measure intracellular ROS. Briefly, Jurkat cells were pre-treated with H₂DCF-DA (10 µM) for 30 min at 37 °C and then treated with 10 µM sulfide for 10 min. The treated cells were washed with PBS (–) for twice. A FACSVerse flow cytometer (BD Biosciences) was used to measure fluorescence of DCF. Data were collected and analyzed by using BD FACSuite software.

2.6. Determination of apoptosis

The quantitative determination and differentiation of viable early and late apoptotic cells were carried out using the Annexin V/Dead Cell Apoptosis Kit (Invitrogen) according to the manufacturer's instructions. Briefly, after treatment, cells were harvested and washed twice with ice-cold PBS (–). The cells stained with the Annexin V/PI mixture were analyzed using a FACSVerse flow cytometer (BD Biosciences). Data were collected and analyzed by using BD FACSuite software.

2.7. Determination of caspase-3 activity

The quantitative determination of caspase-3 activity was carried out using the caspase 3 assay kit according to the manufacturer's instructions. Briefly, after treatment, cells were harvested and washed twice with ice-cold PBS (–). The cells were treated by a lysis buffer (50 mM HEPES, pH 7.4, 5 mM CHAPS, 5 mM DTT, 1 mM PMSF) for 20 min on ice. The lysate was clarified by centrifugation for 15 min at 12,000×g. A total of 75 µg of protein was mixed with a caspase-3 assay buffer (20 mM HEPES, pH 7.4, 2 mM EDTA, 0.1% CHAPS, 5 mM DTT) containing 0.2 mM caspase-3 substrate (acetyl-Asp-Glu-Val-Asp-p-nitroanilide, Ac-DEVD-pNA). The release of pNA was monitored at 405 nm by a multimode microplate reader (Infinite M200; Tecan Group Ltd., Switzerland). Values for nanomoles of pNA released were calculated from those A405 values observed using a standard curve ($y = 0.064x + 0.061$, $R^2 = 0.999$).

Table 1
Structure and induction of DNA fragmentation of fifteen kinds of sulfide flavors in Jurkat cells.

FEMA number	Name	Structure	Concentration (μM)			
			5	10	20	50
3860	Methylthioethane		–	–	–	–
2215	Dibutyl sulfide		–	–	–	–
2747	3-(Methylthio)propionaldehyde		–	–	–	–
3201	Methyl propyl disulfide		–	–	–	–
3228	Dipropyl disulfide		–	–	–	–
2720	Methyl 3-methylthiopropionate		–	–	–	–
3343	Ethyl 3-methylthiopropionate		–	–	–	–
3949	2-Methyl-3-(methylthio)furan		–	–	–	–
3161	Furfuryl isopropyl sulfide		–	–	–	–
3238	Difurfurysulfide		–	–	–	–
3674	Ethyl 3-(furfurylthio) propionate		–	–	–	–
3573	Methyl (2-Methyl-3-furyl) disulfide		+	+	+	+
3259	Bis (2-methyl-3-furyl) disulfide		+	+	+	+
3362	Methyl furfuryl disulfide		–	–	–	+
3146	Difurfuryl disulfide		+	+	+	+

FEMA: Flavor and Extract Manufacturers Association of the United States.
DNA fragmentation was induced by sulfides (+) or not detectable (–).

2.8. Statistical analysis

All values are expressed as mean \pm SD. Statistical analysis was performed using the Student's *t*-test. A level of $P < 0.05$ was considered significant in all statistical tests.

3. Results

3.1. Screening of sulfur flavors with antiproliferative effect in Jurkat cells

The 15 sulfur flavors have certain structural characters as shown in Table 1. Some sulfur flavors contain the same number of sulfur atoms but different side groups, such as monosulfides including methylthioethane, dibutyl sulfide, 3-(methylthio) propionaldehyde, methyl 3-methylthiopropionate, ethyl 3-methylthiopropionate, 2-methyl-3-(methylthio) furan, furfuryl isopropyl sulfide, difurfurysulfide and ethyl 3-(furfurylthio) propionate. However, other sulfur flavors contain the same side groups, but a different number of sulfur atoms, such as 2-methyl-3-(methylthio) furan and methyl (2-methyl-3-furyl) disulfide (MMFDS), difurfurysulfide and difurfuryl disulfide (DFDS). Moreover, there is the phenomenon of the existence of isomers, such as MMFDS and

methyl furfuryl disulfide (MFDS), bis (2-methyl-3-furyl) disulfide (BMFDS) and DFDS.

The antiproliferative potential of these sulfur flavors was evaluated in the Jurkat cell line. Treatment with 5–50 μM of monosulfides did not induce the programmed cell death without significant fragmentation of the DNA (see Supplementary Fig. S1). Similar results were obtained when the Jurkat cells were treated with two linear disulfides, methyl propyl disulfide and dipropyl disulfide (see Supplementary Fig. S2). On the contrary, furan-containing sulfur flavors including MMFDS, BMFDS, MFDS and DFDS induced DNA fragmentation to a varying extent in Jurkat cells. A typical DNA-fragmentation pattern was observed when Jurkat cells were treated with BMFDS at 5–10 μM , MMFDS and DFDS at 5–50 μM for 24 h. However, DNA fragmentation was slightly induced by MFDS at 50 μM (Fig. 1 and Table 1). Endonucleolytic cleavage of genomic DNA into oligonucleosomal fragments along with chromatin condensation is indicative of apoptosis.

3.2. Inhibitory effect of four furan-containing sulfur flavors on cell viability in Jurkat cells

The cell viability was further investigated by CCK-8 assay. As shown in Fig. 2, MFDS tested at a concentration of up to 20 μM

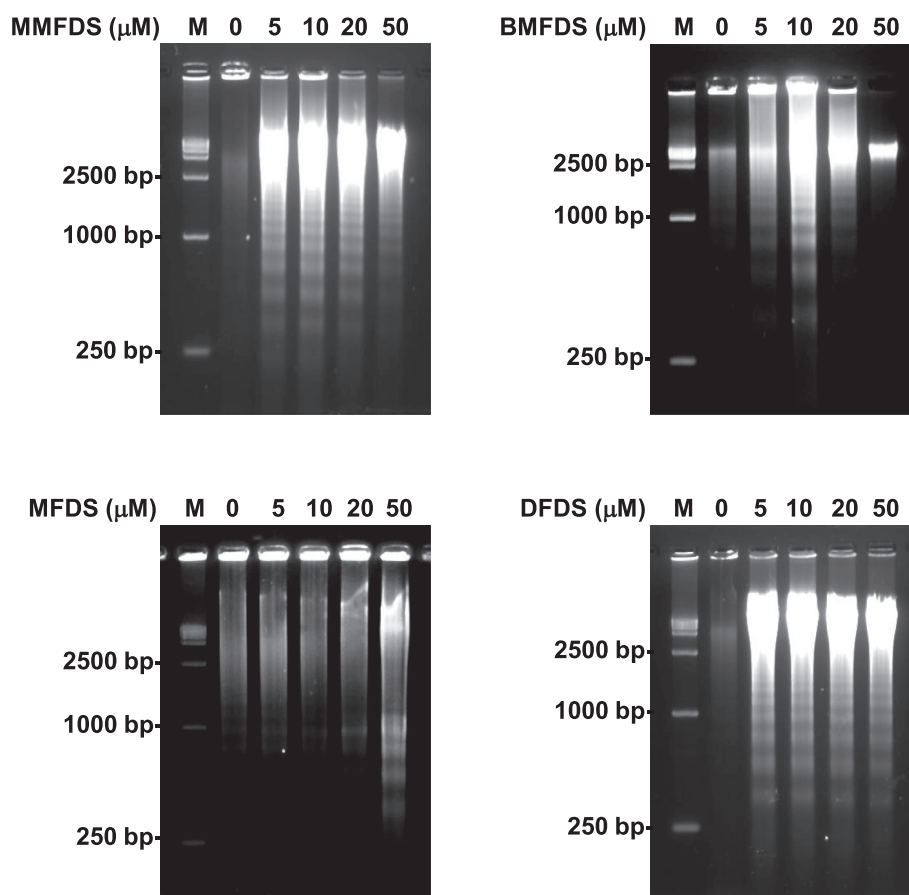


Fig. 1. Induction of DNA fragmentation by furan-containing sulfur flavors in Jurkat cells. Cells (2×10^6) were treated with vehicle (0.1% ethanol), methyl (2-methyl-3-furyl) disulfide (MMFDS), bis (2-methyl-3-furyl) disulfide (BMFDS), methyl furfuryl disulfide (MFDS) and difurfuryl disulfide (DFDS) at 5–50 μM for 24 h. DNA fragmentation was analyzed by electrophoresis. M, molecular weight size marker.

for 24 h resulted in cell viability greater than 80%, which is consistent with DNA fragmentation induced by MFDS. The cell viability in Jurkat cells was inhibited by BMFDS and MMFDS at 5–50 μM in a dose-dependent manner, whereas DFDS gradually decreased cell viability at a dose less than 10 μM . The IC_{50} value indicates a con-

centration of the furan-containing sulfur flavors that caused a 50% reduction in cell viability based on the CCK-8 assay. The IC_{50} value of these four furan-containing sulfur flavors was about 5.8, 10.5 and 26.0 μM for DFDS, BMFDS and MMFDS, respectively. Jurkat cells were significantly less sensitive to the effects of MFDS, as

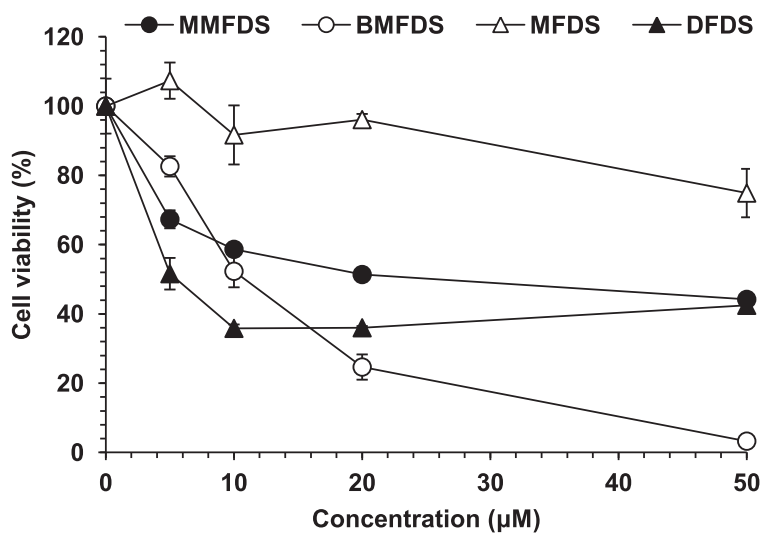


Fig. 2. Effects of furan-containing sulfur flavors on cell viability in Jurkat cells. Cells (2.5×10^4) were treated with vehicle (0.1% ethanol), methyl (2-methyl-3-furyl) disulfide (MMFDS), bis (2-methyl-3-furyl) disulfide (BMFDS), methyl furfuryl disulfide (MFDS) and difurfuryl disulfide (DFDS) at 5–50 μM for 24 h. Cell viability was analyzed by CCK-8 assay. The bar results represent means \pm SD from three or four independent experiments.

the IC_{50} value was higher than 50 μ M. Thus, the viability-reduction effect of these furan-containing sulfur flavors was in the following order DFDS > BMFDS > MMFDS \gg MFDS based on the IC_{50} value in Jurkat cells. These results suggest that, among the selected sulfur flavors, furan-containing disulfide is more favorable to induction of apoptosis in leukemia Jurkat cells, and that DFDS, BMFDS and MMFDS are potential chemopreventive compounds for the inhibition of Jurkat leukemia cells proliferation.

3.3. Induction of intracellular ROS production by methyl (2-methyl-3-furyl) disulfide and bis (2-methyl-3-furyl) disulfide in Jurkat cells

In order to clarify the mechanism of apoptosis induced by DFDS, BMFDS and MMFDS, the intracellular ROS production was determined. After treatment for 10 min, intracellular ROS production was significantly induced by both MMFDS and BMFDS, but not DFDS, at 10 μ M in Jurkat cells. As compared with control (assigned as 1), MMFDS and BMFDS increased the intracellular ROS level by 1.90- and 3.02-fold, respectively (Fig. 3). These results suggest MMFDS and BMFDS may induce apoptosis via ROS production in Jurkat cells. Moreover, ROS production was induced by BMFDS, but not its isomer DFDS, suggesting that the bis (2-methyl-3-furyl) group is more favorable to ROS induction than the difurfuryl group in Jurkat cells.

3.4. Oxidative stress is involved in an antiproliferative effect induced by methyl (2-methyl-3-furyl) disulfide and bis (2-methyl-3-furyl) disulfide in Jurkat cells

In order to clarify the involvement of ROS production in the antiproliferative effect induced by MMFDS and BMFDS in Jurkat cells, the effects of antioxidants, NAC and GSH, on apoptosis induced by MMFDS and BMFDS, were further investigated. As shown in Fig. 4A, NAC and GSH partially suppressed induction of DNA fragmentation MMFDS and BMFDS in Jurkat cells. Unlike other forms of cell death, such as necrosis, apoptosis does not trigger an inflammatory response or result in damage to the surrounding tissue. At the same time, to examine whether necrosis was accompanied by the induced apoptosis in Jurkat cells, the cells

treated with MMFDS and BMFDS for 24 h in the presence or absence of antioxidants were analyzed by an Annexin V-FITC apoptosis kit. Although early apoptotic cells stained with Annexin V-FITC and late apoptotic cells stained with both Annexin V-FITC and PI were enhanced by both MMFDS and BMFDS, the necrotic cells stained only with PI were barely detected. Pre-treatment with NAC or GSH also partially suppressed apoptosis induced by MMFDS and BMFDS (Fig. 4B). Since the DNase responsible for DNA fragmentation has been reported to be directly activated by caspase-3 (Enari et al., 1998), lysates to assay for caspase-3 activity, a marker of cell apoptosis were prepared, by using the colorimetric caspase-3 substrate (Ac-DEVD-pNA). As shown in Fig. 4C, treatment with MMFDS and BMFDS significantly increased caspase-3 activity by 2.3- and 6.1-fold, respectively, in Jurkat cells. Both GSH and NAC partially suppressed the activation of caspase-3 induced by these two sulfides. These results suggest that ROS production and following caspase-3 activation are involved in an antiproliferative effect induced by MMFDS and BMFDS in Jurkat cells.

4. Discussion

In the present study, the antiproliferative effect of furan-containing sulfur flavors in Jurkat cells is indicated, to the authors' knowledge for the first time. Both sulfur atom numbers and side groups in the structure of sulfur flavors determined their modulatory activities on the antiproliferative effect. Three furan-containing sulfur flavors, including DFDS, BMFDS and MMFDS, showed significantly antiproliferative effect in Jurkat cells. Furthermore, the antiproliferation of BMFDS and MMFDS is associated with an increase in intracellular ROS production and subsequent activation of caspase-3 in Jurkat cells.

Among the selected 15 sulfur flavors, all the nine monosulfides had no antiproliferative effect in Jurkat cells. These results are consistent with previous studies showing that allyl and dimethyl monosulfide are devoid of inhibitory effects on cell growth in human colon cancer cells and leukemia cells, respectively (Xiao, Pinto, Gundersen, & Weinstein, 2005; Zhang et al., 2008). The two linear disulfide molecules, methyl propyl disulfide and

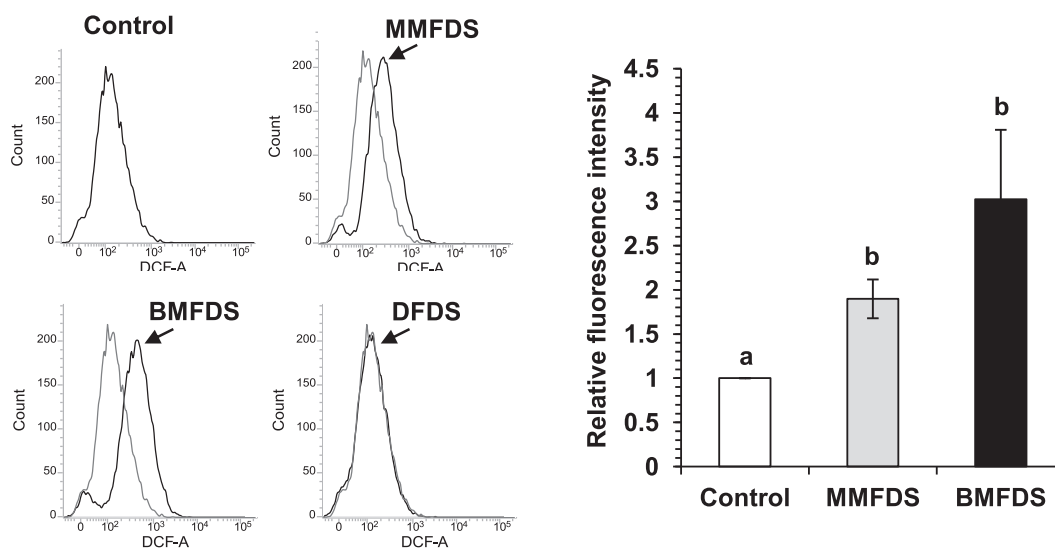


Fig. 3. Intracellular ROS production induced by furan-containing sulfur flavors in Jurkat cells. Cells (2×10^6) were pre-labelled with $H_2DCF-DA$ at 10 μ M for 30 min, and treated with vehicle (0.1% ethanol), methyl (2-methyl-3-furyl) disulfide (MMFDS), bis (2-methyl-3-furyl) disulfide (BMFDS) and difurfuryl disulfide (DFDS) at 10 μ M for 10 min. Intracellular ROS production was analyzed by flow cytometer. The bar results represent means \pm SD from three independent experiments. Different lower case letters indicate significant differences ($p < 0.05$).

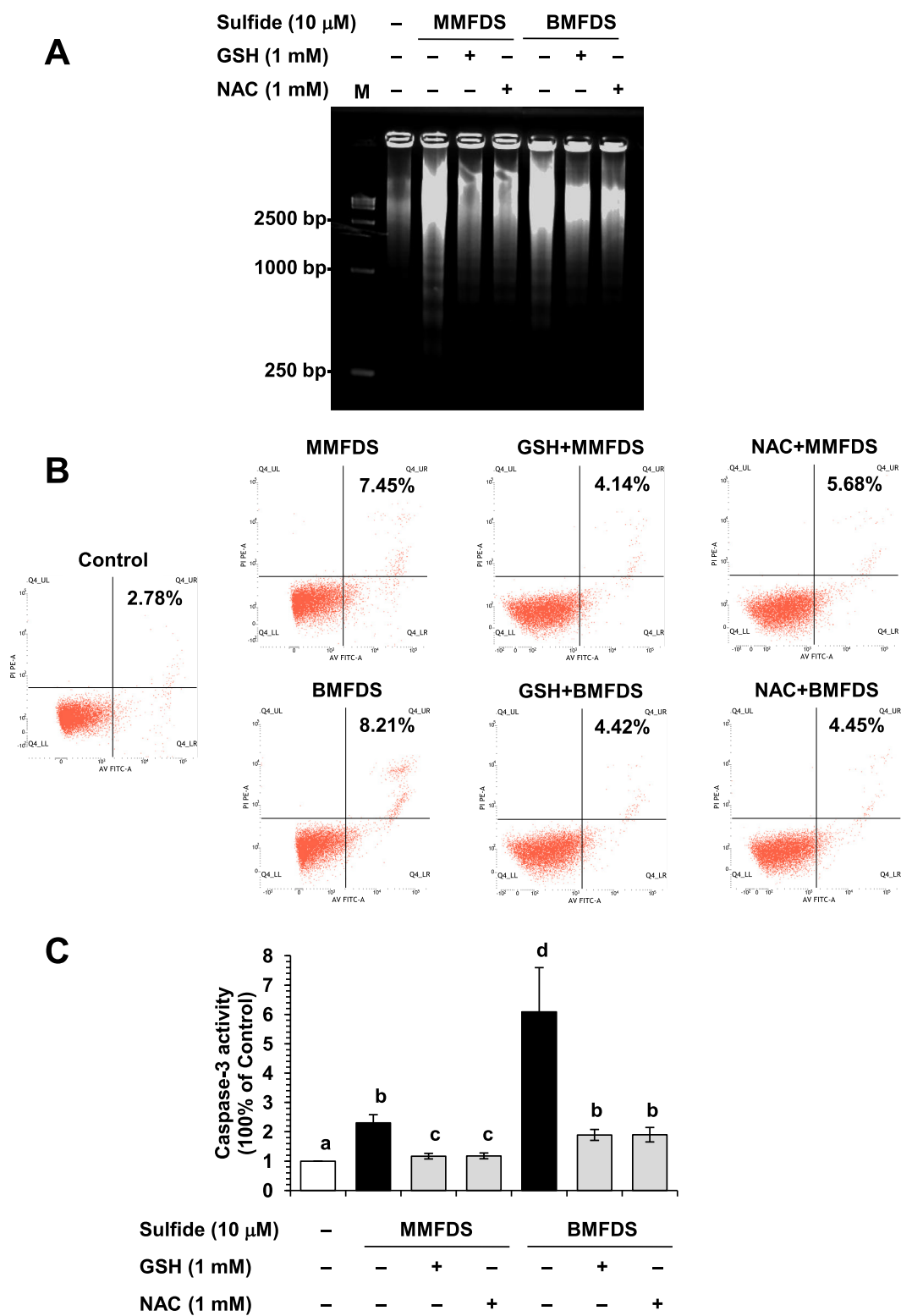


Fig. 4. Involvement of oxidative stress in antiproliferative effect induced by furan-containing sulfur flavors in Jurkat cells. Cells (2 or 5×10^6) were pre-treated with *N*-acetylcysteine (NAC) or glutathione (GSH) at concentration of 1 mM for 30 min, and incubated with methyl (2-methyl-3-furyl) disulfide (MMFDS) or bis (2-methyl-3-furyl) disulfide (BMFDS) at 10 μ M for 24 h. DNA fragmentation was isolated and electrophoresed (A). M, molecular weight size marker. Apoptosis was analyzed by flow cytometer (B). The apoptosis percentage was indicated as the sum of early apoptotic cells stained with Annexin V-FITC and late apoptotic cells stained with both Annexin V-FITC and PI. Total protein was isolated and the caspase-3 activity (C) was analyzed by a caspase 3 assay kit. The bar results represent means \pm SD from three independent experiments. Different lower case letters indicate significant differences ($p < 0.05$).

dipropyl disulfide, also did not induce DNA fragmentation at concentrations of 5 – 50 μ M in Jurkat cells, which is consistent with previous results showing that dipropyl disulfide had no influence

on cell proliferation even at concentration up to 100 μ M in human acute myeloid leukemia cell lines (Merhi, Auger, Rendu, & Bauvois, 2008). These two linear disulfides were found in onion oil

(Takahashi & Shibamoto, 2008), and both of them displayed potency on glutathione S-transferase protein and mRNA induction rat Clone 9 cells (Tsai, Liu, Lin, Chen, & Lii, 2011). Nevertheless, four furan-containing disulfide flavors showed apoptosis induction effect to different degrees, suggesting the presence of the side groups and the number of sulfur atoms mainly account for the differences in activities. According to the viability-reduction results (IC_{50}), the favorable side groups in furan-containing disulfide flavors was in the following order difurfuryl > bis (2-methyl-3-furyl) > methyl (2-methyl-3-furyl) >> methyl furfuryl. Furthermore, the values of IC_{50} for the former three kinds as above ranged from 5 to 26 μ M, which were similar to those of other polysulfides, such as diallyl disulfide (DADS) and DMTS (Xiao et al., 2005; Zhang et al., 2008).

There has been interest in exploiting the anti-proliferative effects of several OSCs and studying their structure–activity relationship. It has been suggested that the number of sulfur atoms in allyl sulfides is an important factor for exhibiting their antiproliferative activity in human colon adenocarcinoma cell lines (Hosono et al., 2005). The cell cycle arrested ability was also increased as the number of sulfur atoms on allyl sulfides increased in skin cancer A375, BCC and human J5 hepatoma cells (Wang, Yang, Hsieh, & Sheen, 2010; Wu, Chung, Tsai, Yang, & Sheen, 2004). Wong et al. (2010) have demonstrated by a structure–activity analysis, that groups adjacent to the disulfide bond are important mediators of tumor-specificity for the modified dysoxysulfone derivatives. They also found all of these compounds with tumor-specific activity are CC-linked α -ester disulfides in leukemic cells. Moreover, it has been reported that alpha-sulfonyl pentyl disulfides have significant promise as antileukaemic agents in various novel disulfides by modifying the structure of dysoxysulfone (Griffiths, Wong, Fletcher, Penn, & Langler, 2005).

Such sulphydryl reductants as NAC and GSH act as radical scavengers, protecting biological systems against oxidative stress. This study found that BMFDS and MMFDS induced ROS production, and that their induction of cell apoptosis was partially counteracted by GSH and NAC, suggesting that redox status plays an important role in suppression of cell viability by these two sulfur flavors. These data are in agreement with our previously published study where we showed that both DMTS and DMTTS induced ROS generation in Jurkat cells (Zhang et al., 2008). The ROS levels induced by DMTS and DMTTS were relatively lower than those induced by BMFDS and MMFDS in Jurkat cells, and the addition of GSH or NAC completely suppressed DMTS and DMTTS-induced apoptosis (Zhang et al., 2008). On the other hand, a lack of DFDS-induced ROS formation in Jurkat cells may indicate that DFDS is not able to activate signaling pathways responsible for the process.

Our previous reports have indicated the significant acceleration effect of several sulfur-containing compounds on degradation of carotenoids under UVA irradiation, in which the effect of DMTTS is mediated by free radicals (Arita et al., 2005; Zhang, Zhu, Nakamura, Shimoishi, & Murata, 2008). This reaction raises the possibility that some sulfur compounds possess pro-oxidant activity. The study demonstrates that diallyl trisulfide (DATS) induced ROS formation in PC-3 prostate cancer cells but decreased ROS formation in PNT1A cells (Antosiewicz, Herman-Antosiewicz, Marynowski, & Singh, 2006; Borkowska, Knap, & Antosiewicz, 2013). Moreover, it has been suggested that DADS may represent an oxidizing agent able to induce oxidative stress-mediated cell death by production of hydrogen peroxide in human leukemia HL-60 cells (Kwon et al., 2002). Oxidative stress also modulates cell proliferation and cell death induced by DADS in human A549 lung cancer cells (Wu, Kassie, & Mersch-Sundermann, 2005).

Caspase-3 is one of the essential molecules for the propagation of the apoptotic signal after exposure to many DNA-damaging agents, and is activated in the apoptotic cell both by extrinsic

(death ligand) and intrinsic (mitochondrial) pathways (Enari et al., 1998; Ghavami et al., 2009). This study found that GSH and NAC significantly suppressed BMFDS and MMFDS-induced caspase-3 activation, indicating that these two sulfur flavors have anti-proliferative effects via caspase-3 pathway in Jurkat cells. These results are consistent with our previous study, which showed that DMTS and DMTTS induced apoptosis via caspase-3 activation pathway in Jurkat cells (Zhang et al., 2008). Other reports have indicated that DATS induced cell cycle arrest and apoptosis via intracellular ROS production and following activation of caspase-3 in both A375 and BCC cells (Wang et al., 2010). Similarly, DADS induced apoptosis in human leukemia HL-60 cells through ROS production and activation of caspase-3 pathway (Kwon et al., 2002). The growth inhibitory effects of DADS on human breast cancer cell lines were coupled with activation of caspase-3 (Nakagawa et al., 2001).

Taken together, our observations indicate that three furan-containing sulfur flavors, namely DFDS, BMFDS and MMFDS, showed antiproliferative effects in Jurkat cells. Moreover, BMFDS and MMFDS-induced ROS generation and subsequent caspase-3 activation initiate apoptosis in Jurkat cells. These findings suggest that these furan-containing sulfur flavors may mediate or contribute to health benefits, and act as potential sources of ingredients to be used in the nutraceutical food industry.

Acknowledgements

This work was supported financially by “The National Natural Science Foundation of China (31201419)”, and “The Major Science and Technology Platform in Universities of Liaoning Province, China ([2011]191)”.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.01.122>.

References

- Agarwal, K. C. (1996). The therapeutic actions of garlic constituents. *Medicinal Research Reviews*, 16(1), 111–124.
- Antosiewicz, J., Herman-Antosiewicz, A., Marynowski, S. W., & Singh, S. V. (2006). C-Jun NH(2)-terminal kinase signaling axis regulates diallyl trisulfide induced generation of reactive oxygen species and cell cycle arrest in human prostate cancer cells. *Cancer Research*, 66(10), 5379–5386.
- Arita, S., Ando, S., Hosoda, H., Sakaue, K., Nagata, T., Murata, Y., et al. (2005). Acceleration effect of sulfides on photodegradation of carotenoids by UVA irradiation. *Bioscience, Biotechnology and Biochemistry*, 69(9), 1786–1789.
- Borkowska, A., Knap, N., & Antosiewicz, J. (2013). Diallyl Trisulfide Is More cytotoxic to prostate cancer cells PC-3 than to noncancerous epithelial cell line PNT1A: a possible role of p66Shc signaling Axis. *Nutrition and Cancer*, 65(5), 711–717.
- Buttke, T. M., & Sandstrom, P. A. (1994). Oxidative stress as a mediator of apoptosis. *Immunology Today*, 15, 7–10.
- Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., & Nagata, S. (1998). A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*, 391, 43–50.
- Ghavami, S., Hashemi, M., Ande, S. R., Yeganeh, B., Xiao, W., Eshraghi, M., et al. (2009). Apoptosis and cancer: Mutations within caspase genes. *Journal of Medical Genetics*, 46, 497–510.
- Griffiths, R., Wong, W. W., Fletcher, S. P., Penn, L. Z., & Langler, R. F. (2005). Novel disulfides with antitumor efficacy and specificity. *Australian Journal of Chemistry*, 58(2), 128–136.
- Guajardo-Flores, D., Gutiérrez-Urbe, S. O., & Serna-Saldívar, J. A. (2013). Evaluation of the antioxidant and antiproliferative activities of extracted saponins and flavonols from germinated black beans (*Phaseolus vulgaris* L.). *Food Chemistry*, 141, 1497–1503.
- Hosono, T., Fukao, T., Ogihara, J., Ito, Y., Shiba, H., Seki, T., et al. (2005). Diallyl trisulfide suppresses the proliferation and induces apoptosis of human colon cancer cells through oxidative modification of beta-tubulin. *The Journal of Biological Chemistry*, 280(50), 41487–41493.

- Kubec, R., Krejčová, P., Mansur, L., & García, N. (2013). Flavor precursors and sensory-active sulfur compounds in Alliaceae species native to South Africa and South America. *Journal of Agricultural and Food Chemistry*, 61(6), 1335–1342.
- Kwon, K. B., Yoo, S. J., Ryu, D. G., Yang, J. Y., Rho, H. W., Kim, J. S., et al. (2002). Induction of apoptosis by diallyl disulfide through activation of caspase-3 in human leukemia HL-60 cells. *Biochemical Pharmacology*, 63(1), 41–47.
- Le Bon, A. M., & Siess, M. H. (2000). Organosulfur compounds from Allium and the chemoprevention of cancer. *Drug Metabolism and Drug Interactions*, 17, 51–79.
- Liu, Y. P., Chen, H. T., Yin, D. C., & Sun, B. G. (2010). Synthesis and odor evaluation of five new sulfur-containing ester flavor compounds from 4-ethylthioacetic acid. *Molecules*, 15(8), 5104–5111.
- Lv, J., Yu, L., Lu, Y., Niu, Y., Liu, L., Costa, J., et al. (2012). Phytochemical compositions, and antioxidant properties, and antiproliferative activities of wheat flour. *Food Chemistry*, 135(2), 325–331.
- McGorin, R. J. (2011). The significance of volatile sulfur compounds in food flavors. In M. C. Qian, X. Fan, & K. Mahattanatawee (Eds.), *Volatile sulfur compounds in food* (pp. 3–31). Washington, DC: ACS Symposium Series 1068, ACS Books.
- McWhinney, S. R., Goldberg, R. M., & McLeod, H. L. (2009). Platinum neurotoxicity pharmacogenetics. *Molecular Cancer Therapeutics*, 8(1), 10–16.
- Merhi, F., Auger, J., Rendu, F., & Bauvois, B. (2008). Allium compounds, dipropyl and dimethyl thiosulfonates as antiproliferative and differentiating agents of human acute myeloid leukemia cell lines. *Bioinformatics Targets & Therapy*, 2(4), 885–895.
- Milner, J. A. (2001). Recent advances on the nutritional effects associated with the use of garlic as a supplement. *The Journal of Nutrition*, 131, 1027S–1031S.
- Minami, M., Matsumoto, S., & Horiuchi, H. (2010). Cardiovascular side-effects of modern cancer therapy. *Circulation Journal*, 74(9), 1779–1786.
- Mouhib, H., Van, V., & Stahl, W. (2013). Sulfur-containing flavors: Gas phase structures of dihydro-2-methyl-3-thiophenone. *The Journal of Physical Chemistry A*, 117(30), 6652–6656.
- Nakagawa, H., Tsuta, K., Kiuchi, K., Senzaki, H., Tanaka, K., Hioki, K., et al. (2001). Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines. *Carcinogenesis*, 22, 891–897.
- Ozben, T. (2007). Oxidative stress and apoptosis: impact on cancer therapy. *Journal of Pharmaceutical Sciences*, 96(9), 2181–2196.
- Perez-Cacho, P. R., Mahattanatawee, K., Smoot, J. M., & Rouseff, R. (2007). Identification of sulfur volatiles in canned orange juices lacking orange flavor. *Journal of Agricultural and Food Chemistry*, 55(14), 5761–5767.
- Polverino, A. J., & Patterson, S. D. (1997). Selective activation of caspases during apoptotic induction in HL-60 cells. Effects of a tetrapeptide inhibitor. *Journal of Biological Chemistry*, 272, 7013–7021.
- Poornima, P., Quency, R. S., & Padma, V. V. (2013). Neferine induces reactive oxygen species mediated intrinsic pathway of apoptosis in HepG2 cells. *Food Chemistry*, 136(2), 659–667.
- Revilla, E., Santa-María, C., Miramontes, E., Candiracci, M., Rodríguez-Morgado, B., Carballo, M., et al. (2013). Antiproliferative and immunoactivatory ability of an enzymatic extract from rice bran. *Food Chemistry*, 136(2), 526–531.
- Ruther, J., & Baltes, W. (1994). Sulfur-containing furans in commercial meat flavorings. *The Journal of Agricultural and Food Chemistry*, 42, 2254–2259.
- Sun, B., Tian, H., Zheng, F., Xie, J., & Liu, Y. (2007). Current status and prospects of sulfur-containing flavor compounds in China. IFEAT International Conference in Budapest, Hungary, Pages 24–35 in the printed Conference Proceedings.
- Takahashi, M., & Shibamoto, T. (2008). Chemical compositions and antioxidant/anti-inflammatory activities of steam distillate from freeze dried onion (*Allium cepa* L.) sprout. *Journal of Agricultural and Food Chemistry*, 56, 10462–10467.
- Thomson, M., & Ali, M. (2003). Garlic [*Allium sativum*]: A review of its potential use as an anti-cancer agent. *Current Cancer Drug Targets*, 3(1), 67–81.
- Thomas, M. B., & Paul, A. S. (1994). Oxidative stress as a mediator of apoptosis. *Immunology Today*, 15, 7–10.
- Tian, H., Sun, B., & Huang, M. (2006). Syntheses and odor characteristics of 1-alkylthio-2-butanethiols, 1-alkylthio-2-butanols and their derivatives. *Perfumer & Flavorist*, 31(1), 32–34.
- Tsai, C. W., Liu, K. L., Lin, C. Y., Chen, H. W., & Lii, C. K. (2011). Structure and function relationship study of allium organosulfur compounds on upregulating the Pi class of glutathione S-transferase expression. *Journal of Agricultural and Food Chemistry*, 59 (7), 3398–3405.
- Wang, H. C., Yang, J. H., Hsieh, S. C., & Sheen, L. Y. (2010). Allyl sulfides inhibit cell growth of skin cancer cells through induction of DNA damage mediated G2/M arrest and apoptosis. *Journal of Agricultural and Food Chemistry*, 58, 7096–7103.
- Wang, X., Martindale, J. L., Liu, Y., & Holbrook, N. J. (1998). The cellular response to oxidative stress: influences of mitogen-activated protein kinase signalling pathways on cell survival. *The Biochemical Journal*, 333, 291–300.
- Willett, W. C. (2002). Balancing life-style and genomics research for disease prevention. *Science*, 296, 695–698.
- Wong, W. W. L., Boutros, P. C., Wasylishen, A. R., Guckert, K. D., O'Brien, E. M., Griffiths, R., et al. (2010). Characterization of the apoptotic response of human leukemia cells to organosulfur compounds. *BMC Cancer*, 10, 351–364.
- Wu, C. C., Chung, J. G., Tsai, S. J., Yang, J. H., & Sheen, L. Y. (2004). Differential effects of allyl sulfides from garlic essential oil on cell cycle regulation in human liver tumor cells. *Food and Chemical Toxicology*, 42, 1937–1947.
- Wu, X. J., Kassie, F., & Mersch-Sundermann, V. (2005). The role of reactive oxygen species (ROS) production on diallyl disulfide (DADS) induced apoptosis and cell cycle arrest in human A549 lung carcinoma cells. *Mutation Research*, 579, 115–124.
- Xiao, D., Pinto, J. T., Gundersen, G. G., & Weinstein, I. B. (2005). Effects of a series of organosulfur compounds on mitotic arrest and induction of apoptosis in colon cancer cells. *Molecular Cancer Therapeutics*, 4, 1388–1398.
- Zhang, G. L., Wu, H. T., Zhu, B. W., Shimoishi, Y., Nakamura, Y., & Murata, Y. (2008). Effect of dimethyl sulfides on the induction of apoptosis in human leukemia Jurkat cells and HL-60 cells. *Bioscience, Biotechnology and Biochemistry*, 72(11), 2966–2972.
- Zhang, G. L., Zhu, B. W., Nakamura, Y., Shimoishi, Y., & Murata, Y. (2008). Structure-dependent photodegradation of carotenoids accelerated by dimethyl tetrasulfide under UVA irradiation. *Bioscience, Biotechnology and Biochemistry*, 72(8), 2176–2183.