Antibacterial Activity of Heated Cabbage Juice, S-Methyl-L-Cysteine Sulfoxide and Methyl Methanethiosulfonate

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ABSTRACT

Autoclaved cabbage juice was inhibitory to growth of Staphylococcus aureus. S-Methyl-L-cysteine sulfoxide (SMCSO), autoclaved either separately, methyl methanethiosulfonate (MMTSO), a thermal breakdown product of SMCSO, completely inhibited growth of S. aureus. MMTSO2 was formed in both autoclaved samples of cabbage juice and aqueous solution of SMCSO. Thus, evidence indicates that the bacterial inhibitory activity in autoclaved cabbage juice was due to heat-induced formation of MMTSO from SMCSO.

Key Words: antibacterial activity, autoclaved cabbage, S-methyl-L-cysteine sulfoxide, methyl methanethiosulfonate, Staphylococcus aureus

INTRODUCTION

ANTIMICROBIAL ACTIVITY in cabbage juice has been the subject of many studies (Dickerman and Liberman, 1952; Kyung and Fleming, 1994a, b; Little and Grubaugh, 1946; Liu et al., 1986; Pederson and Fisher, 1944; Yildiz and Westhoff, 1981) following the initial demonstration of the activity by Sherman and Hodge (1936). Reports concerning inhibitory activity of cabbage have been conflicting. Sherman and Hodge (1936) and Pederson and Fisher (1944) reported that the antimicrobial substance was destroyed by heating. However, Yildiz and Westhoff (1981) reported that filter-sterilized fresh cabbage juice was a better growth medium for Leuconostoc mesenteroides C33 than autoclaved cabbage juice. We observed inhibitory activity in fresh, unheated juice of several cultivars of cabbage (Kyung and Fleming, 1994a) and identified methyl methanethiosulfinate (MMTSO) as the principal antibacterial compound in fresh cabbage (Kyung and Fleming, 1994b). Heating the cabbage before juice extraction prevented formation of the inhibitor(s) in some cultivars, but not others (Kyung and Fleming, 1994a).

MMTSO is formed enzymatically from SMCSO in cabbage juice (Fig. 1; Chin and Lindsay, 1994; Kyung and Fleming, 1994b, Marks et al., 1992). SMCSO, a nonprotein sulfur amino acid, has been reported to be produced in Cruciferae, including cabbage (Arnold and Thompson, 1962; Kyung and Fleming, 1994b; Mae et al., 1971). Marks et al., 1992; Morris and Thompson, 1956; Pederson and Albary, 1969; Syng and Wood, 1956). SMCSO was not growth-inhibitory (Kyung and Fleming, 1994b, 1996). MMTSO was inhibitory to growth of bacteria (Kyung and Fleming, 1994b, Small et al., 1949). MMTSO spontaneously disproportionates into DMDS and MMTSO. (Chin and Lindsay, 1994). MMTSO thus formed was reported to be equal or slightly more inhibitory than MMTSO to growth of bacteria (Small et al., 1949). SMCSO is known to be hydrolyzed into dimethyl disulfide (DMDS) and MMTSO on heating (Fig. 1; Ostermayer and Tarbell, 1960). Thus, our objective was to test the hypothesis that the antibacterial activity of autoclaved cabbage juice was due to formation of MMTSO from SMCSO.

Materials

Methyl methanethiosulfonate (MMTSo), S-methyl-L-cysteine, and hydrogen peroxide were purchased from Sigma Chemical Co. (St. Louis, MO). DMDS was purchased from Aldrich Chemical Co. (Milwaukee, WI). Dimethyl trisulfide (DMTS; Eastman Kodak Co., Rochester, NY) was provided by Dr. R.C. Lindsay, Dept. of Food Science, Univ. of Wisconsin-Madison (Madison, WI). Cabbage juice prepared and previously described by Harris et al. (1992) was used to make an autoclaved cabbage juice. The juice was made by passing quartered cabbage (cv. Green Boy purchased from North Carolina State Farmer’s Market, Raleigh, NC) through a Fritsch (model D comminuting machine, the Fritsch Co., Chicago, IL). The resulting pulp was pressed to extract juice which was then filtered through an ultrafiltration unit with a 1 x 10^-5 Mw cartridge (Amicon Corp., Danvers, MA). The juice was frozen and stored at -20°C in glass containers, then was thawed at room temperature before use. Unheated cabbage juice was extracted by an electrical centrifuge-type juice extractor (Boan, Germany) as described by Kyung and Fleming (1994a). Boiled cabbage juice was made by quartering cabbage and steaming (~100°C) it in an autoclave at atmospheric pressure for 10 min.

Bacterial strain and culture condition

Staphylococcus aureus B31 was obtained from the culture collection maintained by the Food Fermentation Laboratory, USDA-ARS, Raleigh, NC. It was stored at -64°C in trypticase soy (TS) broth (BBL Microbiology Systems, Cockeysville, MD) containing 36% glycerol. The frozen stock culture was streaked onto TS agar, and an isolated colony was transferred to nutrient broth (Difco Laboratories, Detroit, MI) before each experiment. A 10 μL (10 x 10^5 cells) aliquot of overnight statically grown culture of S. aureus in nutrient broth was inoculated into 10 mL of nutrient broth in 16 mm x 150 mm glass culture tubes. All growth experiments were done at 30°C. Viable cell numbers were determined as colony-forming units (CFU)/mL by spiral plating (Spiral Biotech, Bethesda, MD) on plate count agar (Difco Laboratories) and incubating at 30°C for 48 hr.

Preparation of S-methyl-L-cysteine sulfoxide (SMCSO)

SMCSO was prepared by oxidizing S-methyl-L-cysteine with peroxide (Gepp and Dun, 1955). SMCSO thus prepared was confirmed by
mass spectrometry (JOEL, HX-110) using fast bombardment with glyc- 
el. The injection port and detector were at 250°C and 3 min, respectively. Samples were injected in the splitless mode.

Silica capillary column (30m × 250 µm, df = 0.25 mm, Costar bottle filter, Costar Corp., Cambridge, MA), and diluted to 0, 5, 10, 15, 20 ppm with autoclaved nutrient broth. Separation and identification of MMTSO2, MMTSO, DMDS, DMTS. Electron impact ionization (potential 70 eV) was used and the mass spectra. 1-Cyano-3-methylthiopropane was identified by com-
paring experimental with published mass spectra (Spencer and Daxen- 
bichler, 1980).

RESULTS & DISCUSSION

Growth of S. aureus in autoclaved cabbage juice

Autoclaved cabbage juice undiluted or diluted with distilled water to 60% juice completely inhibited growth of S. aureus (Fig. 2). When the inhibitory autoclaved cabbage juice was di-
luted further (40 or 20% cabbage juice), S. aureus grew. Growth rates were faster and total cell populations were higher for S. aureus at lower concentrations of autoclaved juice (Fig. 2). S. aureus grew well in boiled juice but not in unheated juice. This indicated that the inhibitory compound (MMTSSO) in unheated juice was destroyed during boiling and that an inhibitory com-

Growth inhibition by autoclaved SMCSO

Growth of S. aureus was completely inhibited when SMCSO was autoclaved together with nutrient broth, and when its con-
centration was >0.075%. Autoclaved SMCSO at 0.025% in nu-

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SMCSO was added before autoclaving to TS broth which contained more nutrients than nutrient broth. 0.2% SMCSO was necessary to completely inhibit the growth of *S. aureus* (data not shown). We therefore assumed that SMCSO reacted with substances in nutrient broth or TS broth either before or after it was degraded into growth inhibitory compounds. It could also be that TS broth contained substances which inhibit the reaction that results in formation of MMTSO₂, so a higher concentration of SMCSO was needed to provide enough product for notable growth inhibitory effect.

**Growth inhibition of MMTSO₂**

Ten ppm (0.001%) MMTSO₂ in nutrient broth completely inhibited growth of *S. aureus* (Fig. 5). In that case, the number of surviving bacteria was reduced to an undetectable level between 8 and 16 hr incubation. Five ppm MMTSO₂ slightly inhibited growth of *S. aureus*. Reported minimum inhibitory concentrations (MIC) of MMTSO₂ for *S. aureus* were 5 ppm in beef extract broth (15 hr at 37°C; Small et al., 1949) and 50 ppm in TS broth (48 hr at 30°C; Kyung and Fleming, 1996). Since the MIC in our experiment was 10 ppm in nutrient broth, its antibacterial activity seems to be very much dependent on the test medium.

**Presence of MMTSO₂ in autoclaved cabbage juice and in SMCSO solution**

Both autoclaved cabbage juice (antibacterial and non-antibacterial) and autoclaved SMCSO solution contain MMTSO₂ (Fig. 6).
DMTS inhibited growth of sulfoxide (DMTS), and other unidentified compounds. DMDS and products were: MMTSO, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and other unidentified compounds. DMDS and DMTS inhibited growth of S. aureus B31 very slightly, with an MIC of >500 ppm (Kyoung and Fleming, 1996). MMTSO, the principal antibacterial compound in autoclaved cabbage, was found in small quantities both in growth inhibitory and uninhibitory heated cabbage juice. The Michaelis-Menten constant (Ki) of MMTSO for S. aureus was 50 ppm in TS broth (Kyoung and Fleming, 1996). These compounds other than MMTSO found in cabbage juice may exert inhibition action on bacterial growth. 1-Cyano-2,3-epithiopropane (peak 4 of Fig. 6A), a sinigrin degradation product, was not antimicrobial (Kyoung and Fleming, unpublished data). However, we believe that MMTSO, a major thermal degradation product of SMCSO, is the most important antibacterial compound in autoclaved cabbage juice.

CONCLUSIONS

The antibacterial activity of autoclaved cabbage juice is hypothesized to be due to MMTSO, thermally generated from SMCSO, a naturally occurring amino acid in Cruciferae. The presence of MMTSO in autoclaved cabbage juices and autoclaved SMCSO solution was demonstrated. Growth inhibitory cabbage juice had more MMTSO than uninhibitory cabbage juice. Suggesting more SMCSO was present in inhibitory than in uninhibitory juice. SMCSO appears to be the precursor of the antibacterial compound in autoclaved cabbage juice (MMTSO), as was previously shown to be the case with uninhibited cabbage juice (MMTSO).

REFERENCES


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