WASABI, JAPANESE HORSERADISH, AND HORSERADISH

Relationship between Stability and Antimicrobial Properties of Their Isothiocyanates

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Wasabi, Japanese horseradish, and horseradish are used as spices mainly due to their strong pungency and antimicrobial action. When the cell membrane of wasabi or horseradish is broken, the isothiocyanates are formed by the myrosinase-assisted hydrolysis of thioglucosides. However, the isothiocyanates are unstable when in contact with water. The stability of 14 isothiocyanates in aqueous methanol was more affected by temperature than by pH. Furthermore, the antimicrobial properties of these isothiocyanates against four species of bacteria and two species of fungi followed a reverse trend in comparison with their stability. However, the inhibitory effects of these isothiocyanates were not the same for growing cells of S. mutans.

INTRODUCTION

It is well known that the thioglucosides involved in cruciferous vegetables are hydrolyzed by the action of myrosinase to form volatile compounds, including isothiocyanates, thiocyanates and nitriles, among others (Nijssen et al., 1996; Harbone and Baxter, 1993; Kjaer et al., 1953; Mazza, 1984; Kumagai et al., 1994). Among the volatile compounds, isothiocyanates are recognized as being responsible for the characteristic odor and pungency of such vegetables (Ina et al., 1981; Masuda et al., 1996). In general, the isothiocyanates are unstable when allowed to stand in contact with water. Allyl isothiocyanate was gradually decomposed in aqueous solution and produced a garlic-like odor (Kawakishi and Namiki, 1969). In addition, the degradation of the essential oils of wasabi and horseradish in aqueous methanol solution has been studied (Ina et al., 1981; Ina et al.,
1981). However, the stability of each isothiocyanate in wasabi and horseradish under various conditions has not yet been reported.

Wasabi and horseradish are well known to exert antimicrobial actions. The inhibitory effects of the essential oil obtained from wasabi and allyl isothiocyanate, the main component in wasabi, against many species of bacteria using minimum inhibitory concentration (MIC) values have been reported (Inoue et al., 1983). The inhibitory effects of allyl isothiocyanate against different species of bacteria were also studied (Kanemaru and Miyamoto, 1990; Tokuoka and Isshiki, 1994). Furthermore, the antimicrobial action of methyl-, ethyl-, benzyl-, and 2-phenethyl isothiocyanate, involved in horseradish, against some bacteria and fungi (Forter, 1940; Kleese and Lukoschek, 1955; Kojima and Ogawa, 1971), as well as a series of alkyl- and aryl isothiocyanates against some fungi was reported (Drobinica et al., 1967a,b; Mckay et al., 1959; Lien et al., 1968). However, the antimicrobial action of ω-alkenyl- and ω-methylthioalkyl isothiocyanates, the characteristic odor compounds in wasabi and horseradish, respectively, has not been studied.

*S. mutans* is known as the primary causative agent of dental caries in humans (Hamada et al., 1984). The water-insoluble glucan, which is a highly adherent substance, has been reported to be synthesized from *S. mutans* and sucrose (Koga et al., 1986). It has become apparent that *S. mutans* is fixed on the surface of a tooth through the glucan and produces organic acids by metabolism. Benzyl isothiocyanate was found to inhibit the growth and the acid product ability of *S. mutans* (Al-Bagieh and Weinberg, 1988). In addition, allyl isothiocyanate has been reported as an antimicrobial agent against carcinogenic streptococci (Haas, 1976). However, a detailed study of the other isothiocyanates except for benzyl- and allyl isothiocyanate has not yet been made.

This study focuses on the evaluation of the stability and the antimicrobial properties of the isothiocyanates in wasabi and horseradish against four species of bacteria and two species of fungi. Furthermore, the relationship between the stability and antimicrobial properties is presented. The relationship between the stability of the isothiocyanates and the inhibitory effects of selected isothiocyanates on sucrose dependent adherence by growing cells of *S. mutans* is also reported.

**EXPERIMENTAL**

Four ω-alkenyl isothiocyanates, except allyl isothiocyanate, were prepared by isomerization of their corresponding ω-alkenyl thiocyanates (Masuda et al., 1990). Five ω-alkenyl isothiocyanates were converted to their corresponding ω-methylthioalkyl isothiocyanates (Harada et al., 1995). The other isothiocyanates were purchased from commercial sources and purified by vacuum distillation.

The stability of the isothiocyanates was determined as follows. Each isothiocyanate (0.02 mmol) was introduced into 1.4 ml of methanol and 0.3ml of water; the pH was adjusted to 2, 4, 7, and 9 with concentrated hydrochloric acid, acetic acid, distilled water, and sodium carbonate, respectively. The solution was allowed to stand at different temperatures (3, 25, and 50°C). Each solution was extracted with 3 × 50 ml of dichloromethane and concentrated using a rotary evaporator (35°C/300 mm Hg). The residual amounts of isothiocyanates were determined by GC. A Hitachi G-5000 fitted with an FID was used. A DB-1 (30m × 0.25mm i.d.) fused-silica capillary column was employed. The operating conditions were as follows: initial oven temperature, 60°C, then to 250°C at 3°C/min and held for 30 min; injector temperature, 250°C; carrier gas, 0.5 ml/min N₂.