equations reported herein indicated that the sensory properties of canned peaches could be described and predicted from the relative concentrations of the volatile compounds measured by GC analysis.

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LITERATURE CITED

Allen, D. M., *Univ. Ky. Tech. Rep.* **No. 23** (1971).
Broderick, J. J., *Am. Perfum. Cosmet.* **81**, 43 (1966).
Broderick, J. J., *Int. Flav. Food Add.* **6**, 243 (1975).
Chaney, D., Rough, D., Ross, N., Beutel, J., Yeary, E., Horel, L., University of California Leaflet No. 2224 (1976).
Cochran, W. G., Cox, G. M., "Experimental Designs", 2nd ed, Wiley, New York, N.Y., 1957.
CSIRO, Australia, *Food Res. Rep.* No. 118, 119, 120, 122 (1977).
Do, J., Salunkhe, D., Olson, L., *J. Food Sci.* **34**, 618 (1969).
Furia, T., Bellanca, N., "Fenaroli's Handbook of Flavor Ingredients", 2nd ed, Vol. 2, CRC Press, Cleveland, 1975.
Jennings, W. G., Sevenants, M., *J. Food Sci.* **29**, 796 (1964).
Jennings, W. G., Wyllie, S. G., Alves, S., *Chromatographia* **10**, 426 (1977).
Kepner, R. E., University of California, Davis, unpublished data on retention indices, 1974.
Lim, L., "Studies on the Relationship between the Production of Volatiles and the Maturity of Peaches and Pears", M.S. Thesis, University of California, Davis, 1963.
Lim, L., Romani, R., *J. Food Sci.* **29**, 246 (1964).
Maarse, H., Kepner, R. E., *J. Agric. Food Chem.* **18**, 1095 (1970).
Maga, J. A., "Lactones in Foods", *Crit. Rev. Food Sci. Nutr.* **18**, 1 (1976).
Persson, T., von Sydow, E., Åkesson, C., *J. Food Sci.* **38**, 682 (1973).
Romani, R., Jennings, W. G., in: "The Biochemistry of Fruits and Their Products", Vol. 2, Hulme, A. C., Ed., Academic Press, New York, N.Y., 1971, Chapter 12.
Sevenants, M., Jennings, W. G., *J. Food Sci.* **31**, 81 (1966).
Sevenants, M., Jennings, W. G., *J. Food Sci.* **36**, 536 (1971).
Silverstein, R. M., Bassler, G. C., "Spectrometric Identification of Organic Compounds", 2nd ed, Wiley, New York, N.Y., 1967.
Spencer, M. D., M. S. Thesis, University of California, Davis, 1977.
Stenhagen, E., Abrahamsson, S., McLafferty, F. W., "Registry of Mass Spectral Data", Wiley, New York, N.Y., 1974.
Ter Heide, R., *J. Chromatogr.* **129**, 143 (1976).
Yabumoto, K., Ph.D. Dissertation, University of California, Davis, 1976.

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# Lysine Sulfite, a Novel Versatile Salt

Keisuke Kawashima,* Hiroshi Itoh, and Ichiro Chibata

L-Lysine sulfite consisting of 2 mol of L-lysine and 1 mol of sulfurous acid was obtained in a crystalline form by treating L-lysine base with sulfurous acid. Lysine sulfite was much more stable than lysine monohydrochloride against nonenzymic browning reaction. On the other hand, oxidative degradation of lysine sulfite was investigated in aqueous solution by determination of sulfurous acid content. As a result, lysine sulfite was found to be much more stable than inorganic sulfites.

In most of cereals, the first limiting amino acid is lysine and the protein efficiency ratio of cereals is much improved by supplementing lysine. Thus, supplementation with lysine is practically performed in animal feeds and human foods for the purpose of efficient utilization of vegetable proteins. The literature on lysine fortification has been reviewed extensively (Altschul, 1974; Kaneko et al., 1974; Vaghefi et al., 1974).

Lysine supplementation is generally practiced using lysine monohydrochloride. A part of supplemented lysine is, however, known to be inactivated or destructed due to nonenzymic browning reaction (Jansen et al., 1964). According to the study by Cakirer and Lachance (1975), loss of lysine due to baking bread at 395 ± 0.5 °F for 70 min was 16%. Furthermore, browning reaction is sometimes considered undesirable in consequence of the production of colors and off-flavors. For the chemical control of nonenzymic browning reaction, the addition of inorganic sulfites, such as sodium sulfite and sodium bisulfite, is the only practical approach available at present (McWeeny et al., 1974). These inorganic sulfites are also widely used in food industries for many purposes besides inhibition of browning. Since old times, sulfites have been indispensable as antimicrobial agents or antioxidants in wine production. In recent years, they are used as important additives in the biscuit industry to modify the rheological properties of hard sweet biscuit dough (Wade, 1974). However, sulfites are rather rapidly decomposed by oxidation in aqueous solution. The decomposition occurs more rapidly in hard water than in soft water (Heintze et al., 1974). To improve the undesirable properties described above, the authors tried to prepare a versatile salt consisting of lysine and sulfurous acid (Chibata et al., 1974).

The present paper describes the method of preparation of lysine sulfite and some properties of this novel salt.

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EXPERIMENTAL SECTION

**Materials.** A solution of 8% sulfurous acid was purchased from Katayama Chemicals Co., Ltd., Osaka, Japan. L-Lysine hydrochloride was the product of Tanabe Seiyaku Co., Ltd.

**Analytical Methods.** Lysine was determined on an amino acid analyzer, Hitachi KLA-3B. Sulfurous acid

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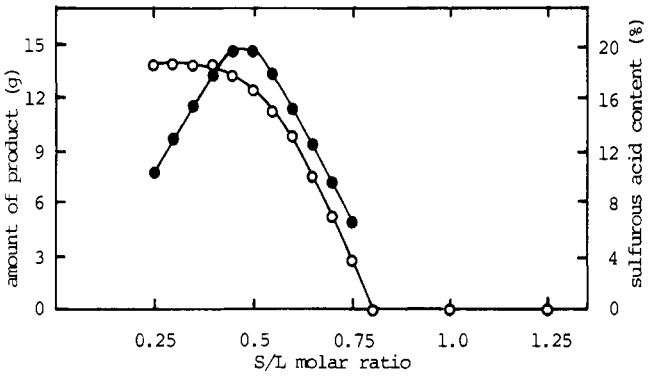


**Figure 1.** Effect of S/L molar ratio on lysine sulfite formation. Into 20 g of lysine base (58.5%) was added 8% sulfurous acid. The S/L molar ratio was varied by altering the amount of sulfurous acid added. Products consisting of lysine and sulfurous acid was separated as described in the text. Solid circles represent the sulfurous acid content of products and open circles represent the amounts of products.

content was assayed by conventional iodometric titration method.

**Treatment of Lysine Base with Sulfurous Acid.** L-Lysine hydrochloride was converted to a free base of lysine using a column of cation-exchange resin, Amberlite IR-120. Finally, lysine base was obtained as an aqueous solution containing 58.5% (w/w) of L-lysine.

Into 20 g of lysine base was added portionwise 8% sulfurous acid with stirring. The molar ratio of sulfurous acid to lysine (the S/L molar ratio) was varied by altering the amount of sulfurous acid added. The solution was cooled in an ice water bath and then four times volume of ethanol was added at once. The mixture was stirred vigorously to precipitate a homogenous mass. This was kept for 3 h at a temperature below 5 °C, and the solid thus formed was separated by filtration. The product was dried over phosphorus pentoxide in vacuo for 24 h at room temperature.

**Preparation of Crystalline Lysine Sulfite.** A crystalline powder (13.03 g) obtained at the S/L molar ratio of 0.5 was carefully recrystallized from hot aqueous methanol to give a colorless brilliant plate, mp 189–192 °C dec. Anal. Calcd for $(C_6H_{14}O_2N_2)_2$–$H_2SO_3$–$0.5H_2O$: C, 37.58; H, 8.14; N, 14.61. Found: C, 38.00; H, 7.96; N, 14.72. The yield was 10.4 g (79.8% based on the crude product). Lysine and sulfurous acid contents were found to be 76.2 and 21.1%, respectively. These data and elementary analysis indicated that the salt was dilysine sulfite with hemihydrate. Heating of the hemihydrate over phosphorus pentoxide at 60 °C for 24 h resulted in formation of anhydrous lysine sulfite, mp 193–195 °C dec; lysine content: 77.9% (calcd: 78.1%), sulfurous acid content: 21.8% (calcd: 21.9%), $[\alpha]^{25}_D$ +8.8 (C = 4, in water).

**Stability Test in Browning Reaction.** Lysine sulfite or lysine hydrochloride (5 mmol based on lysine) and glucose (5 mmol) were dissolved in 10 mL of 0.2 M phosphate buffer (pH 7.0). The solution was sealed up in an ampule and heated at 100 °C for 1 or 2 h. Sodium sulfite and sodium bisulfite were also investigated as inhibitors of browning reaction for comparison. Brown color thus developed was read at 490 nm in a spectrophotometer, Varian Techtron, Model 635D. Solutions with absorbances greater than 1.0 were diluted with distilled water before measuring.

**Stability Test for Oxidative Decomposition of Sulfites.** Erlenmeyer flasks containing 250 mL of 0.1 M sulfites in distilled water, 0.2 M phosphate buffer (pH 7.0) or 0.2 M acetate buffer (pH 4.0) were kept at a constant temperature of 24 ± 1 °C. Sulfurous acid content was determined every other day.

Table I. Effect of Sulfites on Browning Reaction between Lysine and Glucose[a]

| Components | Absorbance at 490 nm 1 h | 2 h |
|---|---|---|
| Glucose + Lys-HCl | 1.002 | 2.730 |
| Glucose + lysine sulfite | 0.018 | 0.130 |
| Glucose + Lys-HCl + $Na_2SO_3$ | 0.045 | 0.167 |
| Glucose + Lys-HCl + $NaHSO_3$ | 0.025 | 0.116 |

[a] Five millimoles of lysine and equimolar glucose dissolved in 10 mL of 0.2 M phosphate buffer (pH 7.0) were heated at 100 °C with or without sulfite (2.5 mmol). Absorbance at 490 nm was measured in a spectrophotometer. Data are averages of duplicate determinations.

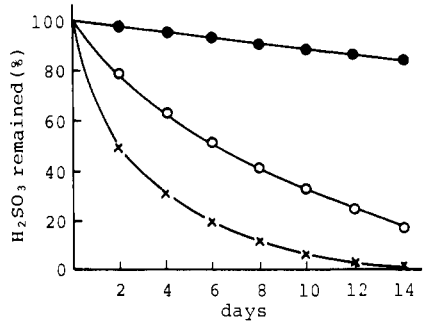


**Figure 2.** Oxidative decomposition of sulfites in aqueous solution. A 500-mL flask containing 250 mL of 0.1 M sulfites in water was kept at 24 ± 1 °C. Sulfurous acid content was determined every other day. Solid circles represent lysine sulfite, open circles represent sodium bisulfite, and crosses represent sodium sulfite.

## RESULTS AND DISCUSSION

Effect of the molar ratio of sulfurous acid to lysine (the S/L molar ratio) on the formation of a sulfurous acid salt of lysine is shown in Figure 1. When the molar ratio was greater than 0.8, no precipitation occurred. So, it is considered that bisulfite type salt is not formed between lysine and sulfurous acid. A product precipitated with the addition of alcohol could be obtained at the S/L molar ratio less than 0.75. Yield of the product increased as the S/L molar ratio decreased from 0.75 to 0.25. Sulfurous acid content of the product also increased with decreasing in the S/L molar ratio until the content reached to the maximum at the ratio of exactly one-half. Below the S/L molar ratio of 0.5, sulfurous acid content decreased linearly as the S/L ratio decreased. The maximum content of sulfurous acid in the product was about 20% which is approximately equal to the theoretical value (21.9%) for a salt consisting of 2 mol of lysine and 1 mol of sulfurous acid. This suggests that lysine formed a stable salt with sulfurous acid as dilysine sulfite. In fact, such a salt could be obtained in a crystalline state as described in the Experimental Section. Interestingly, sulfurous odor of lysine sulfite is much less than that of inorganic sulfites. This may be one of the advantages of using lysine sulfite.

The amount of color developed in the browning reaction between lysine and glucose with and without sulfites is shown in Table I. Data presented in the table are averages of duplicate determinations. In the presence of glucose, lysine hydrochloride caused significant color development while lysine sulfite or lysine hydrochloride plus inorganic sulfites produced little color. It seems worthy of note that lysine sulfite hardly participated in the browning reaction.

Results of the stability test of sulfites against oxidative decomposition both in water and in buffered solutions are

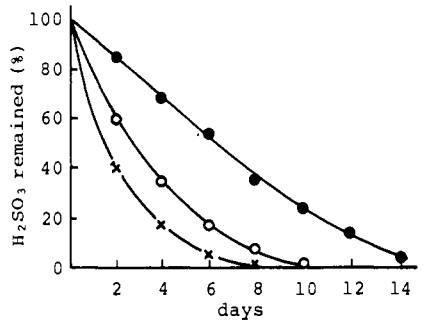


**Figure 3.** Oxidative decomposition of sulfites in phosphate buffer. A 500-mL flask containing 250 mL of 0.1 M sulfites in 0.2 M phosphate buffer (pH 7.0) was kept at 24 ± 1 °C. Sulfurous acid content was determined every other day. Solid circles represent lysine sulfite, open circles represent sodium bisulfite, and crosses represent sodium sulfite.

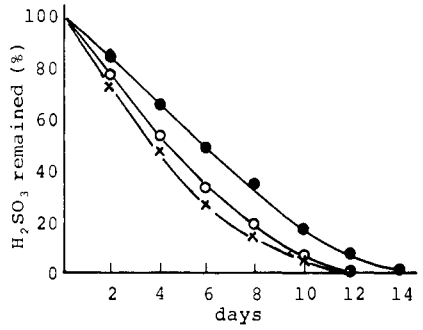


**Figure 4.** Oxidative decomposition of sulfites in acetate buffer. A 500-mL flask containing 250 mL of 0.1 M sulfites in 0.2 M acetate buffer (pH 4.0) was kept at 24 ± 1 °C. Sulfurous acid content was determined every other day. Solid circles represent lysine sulfite, open circles represent sodium bisulfite, and crosses represent sodium sulfite.

shown in Figures 2, 3, and 4. As shown in Figure 2, lysine sulfite is extremely stable in aqueous solution. After 14 days, remaining sulfurous acid content of lysine sulfite solution was more than 80% of the initial amount, while the contents in sodium bisulfite and in sodium sulfite were only 20% and almost 0%, respectively. In the buffered solutions, sulfites were oxidized more rapidly than in aqueous solution (see Figures 3 and 4). Also in this case, lysine sulfite was most stable, followed by sodium bisulfite and then sodium sulfite. However, the difference of stabilities of these salts became smaller at the lower pH. It may be speculated that lysine sulfite is liable to liberate sulfurous acid moiety at the lower pH.

In conclusion, lysine sulfite is much more stable than lysine hydrochloride in the browning reaction. Also the sulfite of this form is more stable than inorganic sulfites in the oxidative decomposition. Although further extensive studies should be done for the practical applications of lysine sulfite, the salt seems to be very versatile and promising. The application of lysine sulfite for food processing, especially for lysine fortified biscuit, seems advantageous. In the pharmaceutical field, sulfites are added in parenteral amino acid infusion to prevent oxidative deterioration. Further, amino acid infusion containing no sodium ion is recommended for some patients with renal disease. Lysine sulfite seems suitable as sulfurous acid source for such a sodium free parenteral infusion. Of course, lysine sulfite should be subject to the same regulatory considerations as other sulfite derivatives.

LITERATURE CITED

Altschul, A. M., *Nature (London)* **248**, 643 (1974).
Cakirer, O. M., Lachance, P. A., *Bakers' Dig.* **49**, 53 (1975).
Chibata, I., Itoh, H., Kawashima, K., Japanese Patent 47 312 (1974); *Chem. Abstr.* **81**, p105977z (1974).
Heintze, K., Braun, F., Fricker, A., *Ind. Obst-Gemueseverwert.* **59**, 452 (1974); *Chem. Abstr.* **82**, 48217w (1975).
Jansen, G. R., Ehle, S. R., Hause, N. L., *Food Technol.* **18**, 367 (1964).
Kaneko, T., Izumi, Y., Chibata, I., Itoh, T., Ed., "Synthetic Production and Utilization of Amino Acids", Wiley, New York, N.Y., 1974, pp 246–251.
McWeeny, D. J., Knowles, M. E., Hearn, J. F., *J. Sci. Food Agric.* **25**, 735 (1974).
Vaghefi, S. B., Makdani, D. D., Mickelson, O., *Am. J. Clin. Nutr.* **27**, 1231 (1974).
Wade, P., *Chem. Ind.*, 721 (1974).

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# Composition of Australian Tea Tree Oil (*Melaleuca alternifolia*)

Greg Swords and G. L. K. Hunter*

Australian tea tree oil (*Melaleuca alternifolia*) was fractionated by column chromatography and analyzed by combined gas chromatography–mass spectrometry. Preparative GLC of selected fractions yielded pure compounds for analysis by infrared and nuclear magnetic resonance spectroscopy. Forty compounds were identified, including viridiflorene which has not been previously reported as occurring in nature.

Tea tree oil is obtained by the steam distillation of *Melaleuca alternifolia*, a small paper-barked tree which grows in natural stands on swampy land along the north coast of New South Wales and the south coast of Queensland, Australia. Tea tree oil has been used in soap perfumes and as a bactericide. It is a good natural source of terpinen-4-ol.

The chemical composition of tea tree oil has been previously investigated by the Instrumental Laboratories of Fritzsche Brothers, Inc.; New York, and the following components were reported (Guenther, 1968): α-pinene, 2.2%; α-terpinene, 7.5%; limonene, 1.0%; 1,8-cineole, 5.6%; γ-terpinene, 17.5%; *p*-cymene, 3.0%; terpinolene, 3.1%; 1-terpinen-4-ol, 44.9%; α-terpineol, 5.2%; aroma-

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