Table VIII. Distribution of ^{14}C after Metabolism of [^{14}C]UDP-glucose by Potato Tissues for 10 h at 20 °C with Cycloheximide (Percent)[a]

fraction	unirradiated	irradiated
CO_2	1.4	1.4
water-insoluble substances	3.9	3.6
phosphate esters	43.5	54.6
sucrose	6.3	7.4
hexoses	5.8	8.7
UDP-glucose, ADP-glucose	20.5	8.3
others	18.6	16.0
total count, dpm	1.29×10^6	1.12×10^6

[a] Each value is a mean of four measurements from four potato tubers. The disks were prepared immediately after irradiation followed by the incubation with cycloheximide in 50 mM phosphate buffer for 24 h at 20 °C, and then the radiolabeled compound was added.

heximide, while that in the irradiated tissues was reduced to the level in the unirradiated ones. It is indicated that the accelerated synthesis of sucrose requires protein synthesis in the irradiated potatoes. It is suggested that the enzymes of which the activities are enhanced by irradiation such as sucrose phosphate synthase and sucrose synthase are rapidly synthesized in irradiated potatoes, which is consistent with the results reported by Nair (1969) in that the synthesis of protein (asparagine synthetase) was accelerated in irradiated potato tubers.

CONCLUSION

The results in this study and the previous work (Hayashi and Kawashima, 1983; Hayashi et al., 1984) suggest that sucrose phosphate synthase plays a more important role in the sucrose accumulation in irradiated potatoes as compared with sucrose synthase. The accelerated synthesis of sucrose phosphate synthase is one of the important physiological changes responsible for the accumulation of sucrose in irradiated potatoes. The reduced breakdown of sucrose and the increased degradation of starch also play a role in the sucrose accumulation. It is concluded that γ-irradiation brings about various physiological changes that together contribute to the accumulation of sucrose in irradiated potato tubers.

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Formation of *N*-Nitrosodimethylamine in Korean Seafood Sauce

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Traditional Korean kimchi prepared from several combinations of vegetables and seafood sauces has been examined for the presence of volatile nitrosamines. Except for trace levels in some samples, no nitrosamines were detected in untreated kimchi and, in most cases, only very low levels of *N*-nitrosodimethylamine (NDMA) could be detected following reaction of the kimchi with acidic nitrite. Kimchi prepared with shrimp sauce or anchovy sauce, however, was found to contain parts-per-million levels of NDMA after treatment with acidic nitrite. Virtually all of this nitrosamine appeared to arise from the fermented shrimp sauce or anchovy sauce used in the preparation of the kimchi. Formation of NDMA in fermented shrimp sauce was effectively inhibited by the addition of ascorbic acid prior to treatment with nitrite.

Kimchi, an important traditional food in Korea, is prepared from salted radish or Chinese cabbage by the addition of fermented anchovy or shrimp sauce along with seasonings and spices, e.g., red pepper powder, garlic, or ginger. In some areas fish or oysters are added to bring out the traditional local flavor. Kimchi prepared near Seoul usually contains shrimp sauce while that prepared in more southern areas tends to contain anchovy sauce. A previous investigation, into the changes in composition and properties of kimchi during preparation, led to evidence suggesting that *N*-nitroso compounds might form at some stages during this process (Kim et al., 1984). This evidence included high initial levels of nitrate followed by a gradual decrease in nitrate concentration, along with decreasing pH, decreasing concentrations of ascorbic acid,

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and increasing levels of secondary amines, including dimethylamine, during the course of preparation of kimchi, which generally takes approximately 10 weeks. The decrease in nitrate concentration is not accompanied by an increase in nitrite concentration; if nitrite is formed, it is apparently rapidly consumed, perhaps by reaction with ascorbic acid or with amino acids. Analysis of several preparations of kimchi failed to reveal significant concentrations of volatile *N*-nitroso compounds in any of the samples (Kim et al., 1984). Thus, even though conditions may be favorable for nitrosation, there is little nitrosating agent in the mixture. It is now well-known, however, that nitrosation can occur in the stomach (Ohshima and Bartsch, 1984; Sander, 1967) and that there is an endogenous supply of nitrite in humans (Tannenbaum, 1983; Reed et al., 1981; Ruddell et al., 1976; Schlag et al., 1980; Tannenbaum et al., 1979). Additionally, Groenen et al. (1982) have shown that seafood and seafood products, when nitrosated in simulated gastric juice, contained higher levels of volatile nitrosamines than did most foods that they investigated. We have consequently studied the reaction of kimchi and some of its components with added nitrite under conditions typical of normal human gastric juice. The results of these experiments are presented below.

EXPERIMENTAL SECTION

Materials. Commerical, imported kimchi and shrimp and anchovy sauce were purchased at retail stores in the Boston/Cambridge area. In one instance samples of locally prepared kimchi were obtained from the manufacturer. Some samples of kimchi were prepared at home or in the laboratory from fresh vegetables and commercial shrimp or anchovy sauce. Nitrosamines were purchased from various suppliers in the United States and were reagent grade or better. The nitrosamines were free of TEA-positive impurities (see below) and were used as obtained.

Sample Preparation. Samples were prepared for analysis by a standard mineral oil extraction procedure (Hotchkiss et al., 1980; Havery et al., 1978). Briefly, a weighed portion of kimchi or sauce was mixed with mineral oil (e.g., Nujol) and placed in a round-bottom flask fitted with a thermometer and attached, via a liquid nitrogen cooled trap, to a mechanical vacuum pump. The mixture was heated under vacuum (2 torr) to 110–120 °C and kept at this temperature for 30 min. The resulting distillate, collected in the cold trap, was extracted with methylene chloride, and the extracts were dried over sodium sulfate or magnesium sulfate and concentrated to 0.5 or 1 mL. The concentrated extracts were then analyzed directly by GC–TEA. In the nitrosation experiments (Marquardt et al., 1977), the samples (25 g) were treated with NaCl (0.7 g) and $NaNO_2$ (0.2 g) at pH 3 for 1 h in the dark at 25 °C. The reactions were then quenched with 0.3 g of ammonium sulfamate and prepared for analysis as described above. Samples for GC–MS were treated by two passages through silica and C_{18} Sep-PAKS prior to analysis.

Analysis. The concentrated extracts were analyzed for *N*-nitroso compounds on a Varian Model 200 gas chromatograph with a 6 ft × $^1/_8$ in. glass column packed with 10% Carbowax 20M–2% KOH on 80–100 Chromosorb WAW. Detection was by thermal energy analysis (TEA), which is sensitive and highly selective for the *N*-nitroso functionality (Fine et al., 1975). The detection limit for *N*-nitrosodimethylamine (NDMA) was 0.008 ppm in the sample being injected. Mixtures showing positive responses to this detector were irradiated in sealed tubes with germicidal lamps, as described by Doerr and Fiddler (1977), in order to detect false positives. The identity of NDMA in shrimp sauce was confirmed by mass spectrometry. These analyses were done on a Hewlett-Packard Model 5995 gas chromatograph–mass spectrometer with fused silica capillary columns coated with either methyl silicone or Supelcowax 10.

Table I. Nitrosodimethylamine Levels in Sauce and Kimchi before and after Nitrosation[a]

sample	NDMA, μg/kg	source
shrimp sauce 1	ND	factory 1
shrimp sauce 1 + NO_2^-	9000	factory 1
shrimp sauce 2	3.4	factory 2
shrimp sauce 2 + NO_2^-	12600	factory 2
anchovy sauce 1	ND	factory 1
anchovy sauce 1 + NO_2^-	1400	factory 1
anchovy sauce 2	ND	factory 2
anchovy sauce 2 + NO_2^-	1100	factory 2
kimchi + shrimp sauce	ND	factory 1
kimchi + shrimp sauce + NO_2^-	83	factory 1
kimchi + shrimp sauce	ND	factory 2
kimchi + shrimp sauce + NO_2^-	80	factory 2
kimchi + shrimp sauce	0.4	home
kimchi + shrimp sauce + NO_2^-	34	home
kimchi + anchovy sauce	ND	home
kimchi + anchovy sauce + NO_2^-	30	home
kimchi + 4× anchovy sauce	1.3	laboratory
kimchi + 4× anchovy sauce + NO_2^-	400	laboratory
kimchi + 4× shrimp sauce	170	laboratory
kimchi + 4× shrimp sauce + NO_2^-	1600	laboratory

[a] No NDEA or NDBA was detected except for approximately 20 μg/kg of a compound coeluting with NDBA in kimchi + shrimp sauce + NO^{2-} and approximately 3 μg/kg of a compound coeluting with NDEA in kimchi + 4× anchovy sauce. Most samples contained unidentified TEA-positive components other than NDMA, *N*-nitrosodiethylamine, or *N*-nitrosodibutylamine. Reaction conditions were as described under Experimental Section. ND: non detected. NDMA: *N*-nitrosodimethylamine.

Table II. Nitrosodimethylamine in Kimchi Components after Nitrosation[a]

sample	NDMA (μg/kg)
fresh Chinese cabbage	3
3% salted Chinese cabbage	3
3% salted Chinese cabbage + spices[b]	4
3% salted Chinese cabbage + radish + spices	3
fresh Chinese cabbage (100 g) + shrimp sauce (25 g)	3000
shrimp sauce (no nitrite)	3
shrimp sauce (+nitrite)	13000
shrimp sauce + nitrite + 2× ascorbic acid	124
shrimp sauce + nitrite + 3× ascorbic acid	130

[a] Reaction conditions were as described under Experimental Section. [b] Spices = red pepper powder (10 g), garlic (10 g), and ginger (1 g) added to 100 g of Chinese cabbage.

RESULTS AND DISCUSSION

Kimchi is a complex mixture of vegetables and flavorings that contains amines, nitrosating agents, and nitrosation inhibitors, many of which change in concentration during the preparation of the kimchi (Kim et al., 1984). The pH of the mixture, in addition, gradually decreases during the course of preparation to levels (i.e., 3 or 4) that are known to favor nitrosation of secondary amines (Mirvish, 1975). There was then, initially, reason to suspect the formation of *N*-nitroso compounds during the preparation of this food. We have consequently examined the levels of volatile *N*-nitroso compounds in several types of kimchi both before and after treatment with nitrite. The results of these experiments are shown in Tables I and II and can be summarized as follows. First, neither shrimp sauce, anchovy sauce, various components of kimchi, nor kimchi itself contains significant levels of common, volatile nitrosamines, although low levels of TEA-positive substances, with GC retention times longer than those of N-nitrosodibutylamine, were observed in most samples.

Second, all samples that were examined were found to contain N-nitrosodimethylamine after treatment with acidic sodium nitrite. The identity of NDMA in shrimp sauce was confirmed by GC–MS; the retention times and the mass spectra were indistinguishable for authentic NDMA and for the compound formed upon nitrosation of shrimp sauce. The major ions in the mass spectrum of an NDMA standard occurred at m/z 74 (100%), 43 (46%), 42 (98%), 40 (12%), and 30 (27%); the mass spectrum of the shrimp sauce nitrosation product had major ions at m/z 74 (100%), 43 (43%), 42 (93%), 40 (7%), and 30 (26%). The levels of NDMA were higher in shrimp sauce than in anchovy sauce, and kimchi that had been prepared with shrimp sauce contained higher levels than kimchi that had been prepared with anchovy sauce. This ranking is consistent with the relative levels of trimethylamine oxide, trimethylamine, dimethylamine, and betaine that have been reported for shrimp sauce, anchovy sauce, and kimchi (Chung and Lee, 1976; Pyeon et al., 1976). The NDMA concentrations in fresh Chinese cabbage with added shrimp sauce were very near those that would be expected from the amount of shrimp sauce that had been added to the preparation, i.e., about 3000 μg/kg NDMA was detected in a nitrosated mixture of about 100 g of Chinese cabbage and 25 g of shrimp sauce while about 13000 μg/kg NDMA was detected in nitrosated shrimp sauce (Table II). The concentrations of NDMA found in nitrosated kimchi (34–83 μg/kg) are essentially the same as those (54–63 μg/kg) reported earlier (Kim et al., 1984) and are much lower than the levels found after nitrosation of a mixture of fresh Chinese cabbage and shrimp sauce, implying that kimchi contains a nitrosation inhibitor, e.g., ascorbic acid (Kim et al., 1974). This idea is supported by the observation (Table II) that the addition of a 2-fold molar excess of ascorbic acid (over nitrite) to shrimp sauce prior to nitrosation almost completely suppressed the formation of NDMA.

Registry No. NDMA, 62-75-9; ascorbic acid, 50-81-7; nitrite, 14797-65-0.

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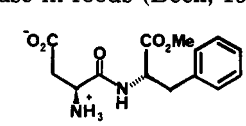
Design of New Artificial Sweeteners Based on Aspartic Acid Amides

George P. Rizzi

The syntheses and taste properties of several new aspartic acid amide sweeteners are described. Infrared and circular dichroism data were used to demonstrate differences in molecular conformations due to hydrogen bonding.

Since the discovery (Mazur et al., 1969) that certain α-amides of L-aspartic acid tasted sweet, much research has been done to establish molecular requirements for the sweet taste (Pavlova et al., 1981). Despite the dozens of sweet compounds synthesized, only one, α-L-aspartyl-L-phenylalanine methyl ester (Aspartame), 1, entered commercial development and recently gained FDA approval for use in foods (Beck, 1978).



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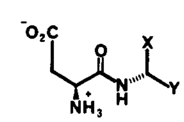

2

Compounds like 1 have attracted much attention because of their potent sweet taste, which when diluted is more similar to sucrose than other artificial sweeteners. Also, these compounds are completely free of bitter aft-

The Procter & Gamble Co., Miami Valley Laboratories, Cincinnati, Ohio 45247.

