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## Chemical Characterization and Sensory Evaluation of a Dietary Sodium-Potassium Fish Sauce

Supshorn Chayovan, Ramu M. Rao,\* Joseph A. Liuzzo, and Mahmood A. Khan<sup>1</sup>

Unviscerated ground fish (flounder and trout with 1.6 and 9.2% fat, respectively) were fermented in a mixture of sodium and potassium salts in different fish to salt ratios. Desired ratios of these salts in the fermented fish sauce were obtained by mixing appropriate volumes of individual salt-based sauce. Chemical analyses included total and ammonia nitrogen, salt, pH, carbonyls, amines, and amino acids. In all sauce samples micromoles per liter concentrations of carbonyls such as butanal, octanol, 2,4-decadienal, 2-undecenal, tetradecanal, etc. and amines (mono-, di-, and trimethylamine) were obtained. Amino acids such as lysine, histidine, arginine, aspartic acid, threonine, etc. ranged in concentrations between  $10^{-4}$  and  $10^{-3}$  mmol/L of sauce. The pH of sauce made from flounder and trout ranged from 5.0 to 6.1 and 4.9 to 5.6, respectively. Sensory analyses indicated that a mixture of NaCl and KCl (NaCl:KCl = 50:50) could provide as replacement for NaCl generally used in fish sauce fermentation.

Fish sauce is a clear brown liquid, rich in NaCl and soluble nitrogen compounds. The concentration of NaCl in these sauces ranges between 25 and 32% in the finished products.

The importance of NaCl in human diets is generally recognized. However, there has been considerable concern about the amount of salt in the diet. Much of this concern centers on questions about dietary sodium and hypertension, or high blood pressure (Dahl, 1972; Meneely, et al., 1957; Meneely and Ball, 1958; Institute of Food Technologists, 1980; Kempner, 1948; Vanderstoep, 1978; Tobias, 1960).

The requirement of NaCl as a taste and flavor enhancer in the human diet is so great that many people find it a hardship to be subjected to a salt-restricted meal. However, numerous publications have reported that naturally occurring KCl can replace sodium salt without affecting the sensory qualities of foods (Frank and Mickelson, 1969; Tucker et al., 1957; Michelsen et al., 1977).

A recent work by Chayovan et al. (1983) has shown that an organoleptically acceptable dietary sodium-potassium fish sauce, which can be used by people who are on a sodium-restricted diet, can be prepared. Therefore, this investigation was undertaken as a continuation of this study to formulate a fish sauce fermented in an optimum mixture of NaCl and KCl salts, to evaluate the sensory acceptability, and to identify and quantify certain chemical and flavor constituents of such a sauce.

A review of literature indicated that commercially produced fish sauces using 100% NaCl are fermented for at least 9 months to develop the desirable flavors. Therefore,

Table I. Formulation of Fish Sauce<sup>a</sup>

sample	KCl, g	KCL:fish	NaCl, g	NaCl:fish	H <sub>2</sub> O, L
Flounder					
1	600	1:3.3			0.5
2	800	1:2.5			0.5
3			600	1:3.3	0.5
4			800	1:2.5	0.5
Trout					
1	600	1:3.3			0.5
2	800	1:2.5			0.5
3			600	1:3.3	0.5
4			800	1:2.5	0.5

<sup>a</sup> Sample weight: 2 kg. Storage temperature: 37 °C.

the present investigation is limited to sensory evaluation and chemical characterization of test samples that were fermented for 6 and 9 months.

### MATERIALS AND METHODS

#### Sample Collection and Preparation of Fish Sauce.

Two varieties of fish—flounder, a lean fish with low fat content (1.6%), and trout, a fatty fish with 9.2% fat—were selected for the preparation of fish sauce. The methods of fish procurement and the preparation of fish sauce were according to the procedures followed by Chayovan et al. (1983). The desired ratios of sodium and potassium salts in the fish sauce were obtained by mixing appropriate volumetric amounts of the sodium- and potassium-based sauces (Table I).

**Chemical Analyses.** Chemical analyses included total nitrogen, ammonia nitrogen, salt content, and pH of 1-, 3-, 6-, and 9-month samples and carbonyls, amines and amino acids of 6- and 9-month samples.

**Total Nitrogen.** Total nitrogen in the fish samples was determined by the Kjeldahl-Gunning modification method as outlined in AOAC (1975).

**Ammonia Nitrogen.** The principle used for the determination of ammonia nitrogen was as follows: Ammonia

Department of Food Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Louisiana State University, Baton Rouge, Louisiana 70803.

<sup>1</sup>Present address: Department of Food and Nutrition, University of Illinois, Urbana, IL 61801.

was released by steam distillation of the sample with  $Mg(OH)_2$  solution. A 0.7-mL sample was pipetted into a steam distillation apparatus and the pipet was rinsed 3 or 4 times with distilled water; 4 mL of 2%  $Mg(OH)_2$  solution was added to the contents of the steam distillation apparatus. The procedure for total nitrogen determination was then followed (AOAC, 1975).

**Salt Content.** The salt content in the fish sauce was determined by the method indicated in AOAC (1975).

**Carbonyls and Amines.** The method for the determination of carbonyls and amines consisted of the following two steps: (1) extraction and (2) isolation, identification, and quantitative measurement of the components by gas chromatography.

The extraction of carbonyl compounds was accomplished by a modified procedure of Fugimaki et al. (1965). The procedure was a batchwise operation in which 500 mL of fish sauce was placed in a three-mouthed distillation flask. The sample was maintained at 120 °C throughout the experiments with the aid of a hemispherical mantle heater. Nitrogen gas was passed through the distillation flask to drive the carbonyl vapors to three successive condensing flasks containing 2,4-dinitrophenylhydrazine reagent (4 g of 2,4-dinitrophenylhydrazine/L of 2 N HCl). All condensing flasks were immersed in a mixture of crushed ice and acetone (-10 °C) containers. Low-boiling carbonyls which might escape the first condensing trap were captured by the two successive traps. The 2,4-dinitrophenylhydrazine solution became turbid after 1 h. To make certain that all the carbonyls from the fish sauce were trapped in the condensing jars, the distillation was continued for another hour at the end of which time the vapors had ceased to appear and an adequate amount of orange precipitate of 2,4-DNPH was produced. The 2,4-DNPH derivative condensates were mixed together in an Erlenmeyer flask which was allowed to stand overnight at room temperature. The following morning, the cloudy solution, or suspension of DNPH's, was extracted with carbon tetrachloride and then with benzene. Both extracts were washed with 2 N HCl and then water. The extracts were dried over powdered anhydrous  $Na_2SO_4$  and filtered. The solvent was removed from the benzene extracts on the steam bath with a jet of nitrogen gas, and residues made to a suitable volume with carbon tetrachloride. The total carbonyls were separated into mono- and dicarbonyls according to the method of Gaddis et al. (1958). The two fractions were then applied to separate columns of alumina (20% hydrated alumina), prepared by adding  $1/4$  in. of powdered anhydrous  $Na_2SO_4$ , and eluted with benzene. After development with ether, the dicarbonyls were eluted with ethanol. Solvents were removed from the monocarbonyl and dicarbonyl fractions at room temperature. The residues were dissolved in 1 mL of carbon tetrachloride and submitted to analysis by gas chromatography.

The procedure used for extraction of amines was a modification of the method of Hughes (1958). Two hundred milliliters of fish sauce samples was mixed with 1 L of 10% trichloroacetic acid. The mixture was continuously stirred with a magnetic stirrer for 5 h. The extract was filtered and the residue was washed with a small amount of solvent. The filtrate was adjusted to pH 5.0 with NaOH, mixed with an equal volume of ethanol, and allowed to stand overnight in a refrigerator. The resulting white precipitate was filtered off, and the filtrate was concentrated at room temperature under reduced pressure (rotary evaporator) until almost all of the ethanol was distilled off. The residue (about 50 mL), after being washed repeatedly with ether to completely eliminate residual fatty materials,

was adjusted to pH 8.0 with  $Na_3PO_4$  and steam distilled for 30 min. The distillate was trapped in two successive flasks containing 200 mL of 2 N hydrochloric acid (150 mL in the first flask and 50 mL in the second flask). The distillate from these two traps was combined and washed with an equal volume of ether and concentrated to dryness at room temperature under reduced pressure. The white powder obtained was submitted to gas chromatography for analysis of carbonyls and amines.

**Isolation, Identification, and Quantitative Measurement of Carbonyls, Amines, and Amino Acids.**

**Carbonyls.** A Perkin-Elmer Model 990 gas chromatographic unit, employing a flame ionization detector (FID), was used. An 8 ft long coil-shaped stainless steel column of  $1/8$ -in. inside diameter was packed with 4% Apizon-L on 60-80-mesh Chromosorb W, AW. The column temperature was programmed from 50 to 280 °C at 3 °C/min. The detector temperature was 300 °C. Nitrogen was used as a carrier gas at a flow rate of 40 mL/min. The sample was injected at 300 °C injection temperature directly into the gas chromatograph, and the carbonyls were identified by comparing the retention times of the peaks with those of standard samples.

**Amines.** The procedure used to isolate, identify, and quantify the amines by gas chromatography was identical with the one used for carbonyls except for column length, column packing materials, and detector, column and injection temperatures. The column was 10 ft long packed with 4% Carbowax 20M-0.8% KOH on 80-100-mesh Chromosorb W, AW. The column temperature was 90 °C (isothermal) with detector and injection temperatures at 150 °C.

**Amino Acids.** The determination of amino acids were achieved by using the method and the equipment setting recommended by Block and Weiss (1956). Analyses were performed on a Beckman Model 116-C amino acid analyzer with 0.2 mL of the hydrolysate being applied to each column. Because tryptophan was destroyed during acid hydrolysis, no determination of this amino acid is reported.

Quantitative analysis of carbonyl, amines, and amino acids was achieved by the Internal Standardization Method (Kobot and Ettore, 1963). The molar concentrations of the components were determined by the relative molar concentration (Preston and Spreckelmeyer, 1971).

**Sensory Evaluation.** The sensory evaluation based on the attributes of flavor, color, and overall acceptability of mouth feel was performed according to the procedures outlined by Chayovan et al. (1983). The sensory data were submitted to standard analysis of variance to test the effects of salt levels (KCl:NaCl percent ratios) and fish varieties (flounder and trout) on the flavor, color, and overall quality. All tests of significance were made at 0.01 and 0.05 levels of probability (Snedecor, 1959).

## RESULTS AND DISCUSSION

Statistical analyses of the sensory data indicated that the fish sauce containing 50% KCl and 50% NaCl was well accepted by the judges. Therefore, chemical analyses were limited to only this sauce mixture. Consequently, sensory results are presented prior to the results of the chemical analyses.

**Sensory Evaluation.** Statistical analyses of the data indicated that salt content (KCl:NaCl ratios) influenced flavor ( $p < 0.01$ ) and overall quality ( $p < 0.05$ ) as shown in Table II. Results in Table III show that the trend toward acceptability of flavor and overall quality terminates at the 60:40 ratio of KCl and NaCl. A rejection of these attributes at higher KCl and NaCl ratios could be due to the known unpleasant bitter taste of potassium salt.

Table II. Analysis of Variance of Acceptability of Flavor, Color, and Overall Quality of Fish Sauce Made by Replacing Various Levels of NaCl with KCl

source	df	flavor m.s.	color m.s.	overall quality m.s.
judge ( <i>J</i> )	9	0.874 <sup>b</sup>	2.200 <sup>b</sup>	3.557 <sup>b</sup>
treatment ( <i>T</i> )	6	80.157 <sup>b</sup>	9.080 <sup>b</sup>	73.675 <sup>b</sup>
salt content ( <i>S</i> )	1	6.914 <sup>b</sup>	0.014	1.728 <sup>a</sup>
fish variety ( <i>F</i> )	1	0.357	0.128	2.057 <sup>b</sup>
<i>T</i> × <i>S</i>	6	0.097	0.080	0.286
<i>T</i> × <i>F</i>	6	0.257	0.561 <sup>a</sup>	0.715 <sup>a</sup>
<i>S</i> × <i>F</i>	1	0.514	0.228	0.057
<i>T</i> × <i>S</i> × <i>F</i>	6	0.097	0.228	0.332
residual	243	0.259	0.235	0.301
corrected total	279	2.015	0.491	2.00

<sup>a</sup> Significant at the 5% level. <sup>b</sup> Significant at the 1% level.

Table III. Treatment Means from Analysis of Variance on the Acceptability of Flavor, Color, and Overall Quality of Fish Sauce Prepared by Various Levels of NaCl:KCl<sup>a</sup>

ratio KCl:NaCl	flavor	color	overall quality
0:100	4.87	4.85	4.80
20:80	4.45	4.87	4.62
40:60	4.02	4.67	4.27
50:50	3.60	4.75	3.90
60:40	3.17	4.32	3.30
80:20	1.57	3.97	2.00
100:0	1.15	3.65	1.25
overall means	3.26	4.44	3.45

<sup>a</sup> Score codes: 1 = least acceptable; 5 = most acceptable. Each mean is the average of 40 observations.

Table IV. Combination Means for Flavor, Color, and Overall Quality of Fish Sauce with KCl and NaCl Mixtures at Two Levels of Salt Concentrations<sup>a</sup>

	low salt (300g/kg of fermented fish)		high salt (400g/kg of fermented fish)	
	trout	flounder	trout	flounder
flavor	3.500	3.342	3.100	3.114
color	4.400	4.500	4.442	4.428
overall quality	3.600	3.457	3.471	3.271

<sup>a</sup> Each mean is the average of 70 observations.

The high-salt concentration and fish variety did not significantly ( $p < 0.05$ ) affect the acceptance on the sauce

color but significantly reduce the flavor ( $p < 0.01$ ) and overall quality ( $p < 0.05$ ). The sauce prepared by fermenting fish at low-salt concentration was more favorable. It is thought that by using as low a salt content as 300 g/kg of fish fermented in making fish sauce is more desirable than the 400 g/kg of fish fermented (Table IV). The interaction of treatment on fish was found to significantly ( $p < 0.05$ ) reduce the color acceptance and overall quality. This probably was due to the high content of fat in the fatty fish which inhibits the sugar-amino browning reaction during fermentation.

**Chemical Analysis.** The sauce samples used in this investigation were drawn 4 times at the end of 1, 3, 6, and 9 months of fermentation. The pH, total nitrogen, ammonia nitrogen, and salt contents are shown in Table V.

The pH of fish sauce from flounder (lean fish) and trout (fatty fish) ranged between 5.0–6.1 and 4.9–5.6, respectively. The pH of both sauces reached their highest levels at the sixth month of fermentation. These observed values agreed closely to those reported by Uyenco et al. (1952) on Vietnamese fish sauce. Kasemsarn (1963) reported that fish with a pH range from 5.0 to 6.5 were optimum for sauce fermentation.

Ammonia nitrogen ranged from 1.05 to 3.62 and 1.25 to 3.34 g/L of sauce, respectively, for flounder and trout. The amount of ammonia increased gradually from the beginning and reached its highest level in both 6-month-old samples. Total nitrogen reached the highest level at the ninth month ranging from 8.1 to 23.77 g/L for flounder and 7.5 to 23.8 g/L for trout.

The salt concentration remained fairly constant in all samples from the beginning of fermentation until the end of the process. This could have been due to the salt concentration having reached equilibrium during the early stage. The salt concentration in the sauce affects the rate of bacterial spoilage and enzyme activities. According to Baxter and Gibbons (1956), those bacteria that could grow over the range from 1 to 20% NaCl would be classified as moderate halophiles. Saisithi et al. (1965) reported that the number of viable bacteria able to grow at salt levels present in fish sauce were low and these microorganisms are unlikely to play a significant role in the solubilization of fish proteins. Velankar (1952) and Kasemsarn (1963) concluded that the protein hydrolysis which occurs during fish fermentation is due to autolytic processes. The proteolytic enzymes which cause autolysis may either come from the viscera or bacteria which may previously exist

Table V. Effect of Fermentation of pH, Ammonia Nitrogen, Total Nitrogen, and Salt Concentrations in Fish Sauces

fermen- tation, months at 37 °C	chemical composition	flounder (low fat)		trout (high fat)	
		low salt <sup>a</sup>	high salt <sup>b</sup>	low salt <sup>a</sup>	high salt <sup>b</sup>
1	pH	5.2	5.0	4.9	5.0
	total ammonia nitrogen, g/L	1.22	1.05	1.25	1.25
	total nitrogen, g/L	8.80	8.10	10.85	7.50
	salt concentration, g/L	255.00	273.00	249.00	275.00
3	pH	5.5	5.7	5.4	5.5
	total ammonia nitrogen, g/L	2.10	2.52	2.20	2.00
	total nitrogen, g/L	13.30	12.90	17.06	14.00
	salt concentration, g/L	252.00	277.00	250.00	274.00
6	pH	5.6	6.1	5.6	5.5
	total ammonia nitrogen, g/L	3.33	3.62	3.34	3.11
	total nitrogen, g/L	22.50	18.50	23.64	19.80
	salt concentration, g/L	252.00	278.00	252.00	276.00
9	pH	5.4	6.1	5.5	5.5
	total ammonia nitrogen, g/L	3.34	3.61	3.30	3.07
	total nitrogen, g/L	23.40	23.77	23.80	20.70
	salt concentration, g/L	250.00	276.00	248.00	270.00

<sup>a</sup> 300 g/kg of fish. NaCl:KCl = 50:50. <sup>b</sup> 400 g/kg of fish. NaCl:KCl = 50:50.

Table VI. Carbonyl Composition of Fermented Flounder and Trout Fish Sauce

carbonyls	$\mu\text{mol/L} (\times 10^{-5})$			
	flounder (low fat) stored		trout (high fat) stored	
	6	9	6	9
	months	months	months	months
butanal	4.4	2.5	3.0	2.5
octanal	5.0	3.3	8.9	5.3
2,4-decadienal	trace	trace	0.8	trace
2-undecenal	49.00	47.00	11.00	80.0
tetradecenal	8.6	9.2	22.0	16.0
hexadecanal	7.8	5.3	4.2	2.2
octadecanal	4.7	1.6	8.4	4.4
octadecenal	trace	0.00000	1.4	trace
octadecadienal	0.0	0.0	trace	trace
total	79.5	68.9	158.0	110.4

Table VII. Amines of Flounder and Trout Fish Sauce Fermented for 6 Months

	$\mu\text{mol/L} (\times 10^{-5})$	
	flounder (low fat)	trout (high fat)
	methyamine	4.2
dimethylamine	6.3	13.7
trimethylamine	38.0	3.1
total	48.5	25.2

on or in the fish prior to the salting period. The heavy concentration of the salt solution evidently causes the sensitivity of bacterial cells to slow down and leave behind the constituent proteolytic enzymes to act upon fish tissues (Tressler and Lemon, 1951). Proteolysis that takes place during fermentation is assumed to yield peptides and amino acids that may undergo a variety of changes including deamination, decarboxylation, and transamination to yield amines, keto acids, ammonia, and carbon dioxide; lipids and phospholipids are further hydrolyzed to flavor components such as volatile and nonvolatile fatty acids and carbonyl compounds (Saisithi et al., 1965).

**Carbonyl Compounds.** The carbonyls (Table VI) found in the samples are believed to be the degradation products from free fatty acids or triglycerides. Evidence relating the flavors in individual carbonyls to specific attributes of fish flavor has been reported by Diemair and Schams (1962), and Diemair (1964). On the basis of these works, Diemair suggested that the carbonyls of low molecular weight are associated with fresh flavor. The "tranig" odor components of fat containing species come mainly from hexanal, heptanal, and hexenal, while the ranacid and "taligen" components belong to the higher constituents of the saturated and unsaturated fatty acids such as nonanal, heptadienal, and decanal. A strongly "metallic" component discussed by Diemair (1964) has many of the properties of oct-1-en-3-one (*n*-amyl vinyl ketone) (Forss, 1963). Diemair and Schams (1962) also isolated a carbonyl component with a "mushroom" odor from stored fish. From its properties this was thought to have a dihydroxypropane skeleton. Forss (1963) reported that there are strong similarities between certain components of fishy flavor in dairy products and those isolated from stored fish by Diemair and Schams (1962). Although none of the carbonyls listed in Table VI seem to possess the characteristic flavor of fish sauce, the mutual interaction of mixing may cause some effects on the development of fish sauce aroma.

**Amines.** Total and individual amounts of volatile amines in all the test samples (Table VII) were extremely low compared to those values generally reported to be present in fish (Kasemsarn, 1963). It is possible that the

Table VIII. Ammonia and Amino Acid Concentrations of Fermented Flounder and Trout Fish Sauce

ammonia/ amino acids	M/L for flounder (low fat) at storage, months, of		M/L for trout (high fat) at storage, months, of	
	6	9	6	9
	ammonia	0.0048	0.0034	0.0064
lysine	0.0031	0.0036	0.0026	0.0033
histidine	0.0008	0.0008	0.0015	0.0008
arginine	0.0007	0.0006	0.0008	0.0006
aspartic acid	0.0019	0.0025	0.0013	0.0018
threonine	0.0010	0.0014	0.0012	0.0016
serine	0.0005	0.0006	0.0006	0.0006
glutamic acid	0.0008	0.0016	0.0012	0.0017
proline	0.0034	0.0036	0.0023	0.0090
glycine	0.0058	0.0055	0.0064	0.0057
alanine	0.0058	0.0049	0.0077	0.0070
cystine	0.0017	0.0010	0.0011	0.0008
valine	0.0028	0.0040	0.0054	0.0060
methionine	0.0021	0.0015	0.0021	0.0015
isoleucine	0.0021	0.0029	0.0026	0.0023
leucine	0.0025	0.0032	0.0036	0.0043
tyrosine	0.0010	0.0007	0.0014	0.0008
phenylalanine	0.0014	0.0015	0.0022	0.0014
total	0.0422	0.0433	0.0504	0.0536

loss of these amines in fish sauce might have occurred during the earlier period of fermentation. It has been reported that volatile amines are produced occasionally through decarboxylation of corresponding amino acids during heat treatment of foods (Weurman and Rooji, 1961). However, this reaction probably did not occur under the present experimental conditions since the fermentation products were not heat treated.

**Amino Acids.** The basic tastes perceptible by the human tongue are restricted to four simple ones: sour (acid), bitter, sweet, and salty. These primary taste sensations are critically supplemented by a large number and variety of odor perceptions registered by the olfactory apparatus (Ingram, 1966). However, the flavor which will be discussed here is restricted mainly to the nonodoriferous constituents which give only basic taste perceptible to the human tongue. It has been suggested by Jones and Murray (1961) that part of the fish flavor is derived from sugar, sugar phosphates, amino acids and peptides, nucleotides and derivatives, organic acids, fats and degradation products of fats, and nitrogenous bases. He has reported that a combined amino acid solution, simulating that of fresh gadoid muscle, possesses a pleasant, sweet-sour, meaty, and yeasty flavor. The amino acids present in 6- and 9-month fermented fish sauce are shown in Table VIII.

No qualitative change was found in the amino acids present in both trout and flounder fish sauce samples from 6- and 9-month fermentation. However, a slight quantitative increase in the 9-month sample was observed. Claims have been made that certain amino acids can occur in fish muscles at such concentrations that they contribute to flavor independently of other constituents. Thus glycine can contribute to sweetness (Amano and Bito, 1951; Hashimoto, 1964) and histidine to meaty character (Simidu et al., 1953a,b). However, this is not always the case. The individual amino acids of gadoid muscles were found to occur below the flavor threshold, but they were readily detectable in a composite simulated extract (Jones and Murray, 1961). Similarly, Konosu et al. (1960) found that a simulated amino acid fraction (which included histidine) was almost flavorless. This group noted a pronounced mutual enhancing a flavor in combination of the amino acid fraction with 5'-monophosphate. In a further study

on fractionated extracts of abalone meat, Konosu and Hashimoto (1964) demonstrated a similar key interrelationship to a meaty character between glutamic acid and adenosine 5'-monophosphate. Komato (1964) obtained similar results with "Uni", the unripe gonad of the sea urchin. Of the amino acid fraction in combination with mononucleotides, he found glycine, valine, alanine, glutamic acid, and, particularly, methionine to be important. The elimination of glycine resulted in an increase in bitterness and a decrease in sweetness.

Such findings tend to confirm the suggestions that amino acids are likely to be important nonodoriferous contributors to the overall flavor of fish sauce.

Experimental evidence obtained by chemical and sensory analyses has shown that mixtures of NaCl and KCl (NaCl:KCl = 50:50) could provide as a possible replacement for common salt (NaCl) generally used in fish sauce fermentation.

**Registry No.** Na, 7440-23-5; NaCl, 7647-14-5; KCl, 7447-40-7; ammonia, 7664-41-7; nitrogen, 7727-37-9; butanol, 123-72-8; octanal, 124-13-0; 2,4-decadienal, 2363-88-4; 2-undecenal, 2463-77-6; tetradecenal, 54264-02-7; hexadecanal, 629-80-1; octadecanal, 638-66-4; octadecenal, 71873-66-0; octadecadienal, 28982-40-3; monomethylamine, 74-89-5; dimethylamine, 124-40-3; trimethylamine, 75-50-3.

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## Indole Glucosinolates in Swede (*Brassica napobrassica* L. Mill)

Roger J. W. Truscott,\* Patricia K. Johnstone, Ian R. Minchinton, and Joseph P. Sang

A new high-performance liquid chromatography (HPLC) procedure for glucosinolate analysis has been used to reinvestigate the glucosinolate content of swede (*Brassica napobrassica* L. Mill), also known as rutabaga. Swede rind was shown to contain four distinct indole glucosinolates: glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin. The desulfo derivative of neoglucobrassicin was purified by HPLC and the 1-methoxyglucobrassicin structure confirmed by a combination of ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry techniques. Thus, two methoxyglucobrassicin isomers, 4-methoxy and 1-methoxy, coexist in the same plant tissue.

The glucosinolate content of cruciferous plants is important in view of their role as flavoring constituents. Once

cellular disruption occurs glucosinolates undergo enzymic hydrolysis with endogenous myrosinase, releasing isothiocyanates and other products which contribute to the flavor of brassica vegetables. The presence of glucosinolates in *Brassica* species is also associated with toxic and

Division of Agricultural Chemistry, Department of Agriculture, East Melbourne, Victoria, Australia 3002.